OHIO STATE UNIVERSITY
JAN 9 1962
LIBRARY

HC

THE TOHOKU JOURNAL OF EXPERIMENTAL MEDICINE

Incorporating

ARBEITEN AUS DEM ANATOMISCHEN INSTITUT
DER KAISERLICH-JAPANISCHEN
UNIVERSITÄT ZU SENDAI
AND
MITTEILUNGEN ÜBER ALLGEMEINE PATHOLOGIE
UND PATHOLOGISCHE ANATOMIE

RECEIVED
JAN 10 1962
HEALTH CENTER LIBRARY

Vol. 71. No. 4, March 25, 1960

EDITORIAL BOARD
T. KATO K. MOTOKAWA M. WADA

WITH COLLABORATION OF

K. AKAZAKI, T. EBINA, T. FUJITA, Y. HAYASHI, S. IINO, T. ISHIBASHI, T. ISHIKAWA, M. ITO, K. IWATSUKI, S.-T. KATSURA, G. KIKUCHI, N. KIRISAWA, Y. KOGA, S. KONDO, T. KUMAGAI, T. KUROKAWA, M. KUROYA, K. KUSHIMA, T. MURAKAMI, M. MUTO, F. NAKAZAWA, S. NASU, S. OSATO, T. SANO, A. SATO, S. SATO, M. SEGI, T. SHIMAUCHI, H. SETO, T. SHINODA, SH. SUGIMURA, T. SUGIYAMA, N. SUWA, T. SUZUKI, E. TAKAHASHI, Y. TAKAHASHI, M. TERASAKA, T. TORIGAI, Y. TUIKI, R. URA, S. YAMAGATA

PUBLISHED BY

THE TOHOKU UNIVERSITY

SENDAI, JAPAN

The Tohoku Journal of Experimental Medicine is an official organ of the Tohoku University for the publication of records of original research in all branches of experimental medicine, but accepts also papers from sources other than the University, domestic as well as overseas, under certain limitations, given below.

Two to three volumes, each containing four numbers, are issued a year. Each volume will consist of approximately 400 pages.

The articles are written in English, French or German.

The subscription price is $5.00, foreign $5.50, per volume, post-paid, and should be sent to the MARUZEN CO., LTD., Tokyo or CHARLES E. TUTTLE CO. RUTLAND, VERMONT, U.S.A.

Papers and correspondence regarding exchange of publications, etc. should be addressed to the Medical Library, Tohoku University, Kita-yoban-tyo, Sendai.

Notice to Contributors

Papers will not be accepted for simultaneous publication or those which have been previously published in the periodicals written in English, French, German or Italian. All articles from Laboratories must be approved by the director of the originating laboratory. Contributions should be ordinarily limited in length to 10 printed pages. Every paper must be accompanied by a brief summary.

The manuscripts should be typewritten in double spacing, and carefully revised before submission to the Editor. The original, not carbon, copy should be sent. Bibliographies should give the name of the author, the name of journal or book (with edition & place of publishing), the year, the volume and the page.

Figures should be ready for photographic reproduction, diagrams should be drawn in Indian ink and plain white or fine blue-lined paper only should be employed ; letters, numbers etc. should be written in pencil. The reduction desired should be clearly indicated on the margin of the drawing. The Journal cannot accept illustrations which when reproduced for printing exceed the size of a Journal page. Authors should indicate on the manuscript the approximate position of the text figures and tables.

All the drawings and tables should be marked with the author's name. They should be packed plate, carefully protected from injury, and insured.

Proofs will not be submitted to authors overseas unless they express a desire to see them, or unless the editors think it ne essary. Overseas authors are therefore particularly reminded of the importance of preparing their manuscripts in such a way that errors need not arise.

Authors receive 100 reprints of articles at cost.

The Tohoku Journal of Experimental Medicine, Vol. 71, No. 4, 1960

The Effect of Hemorrhage on the Noradrenaline and Adrenaline Contents of the Adrenal Gland in the Cat

By

Mituki Ide
(井手 水月)

From the Department of Physiology, Nagasaki University School of Medicine, Nagasaki

(Received for publication, October 28, 1959)

It is well established that adrenal gland of the cat contains considerable amount of noradrenaline together with adrenaline. In an attempt to evaluate the possible role of noradrenaline in adrenal physiology, investigations were undertaken to know how the various kinds of stressful stimuli affect the noradrenaline and adrenaline contents of the adrenal gland. In my previous study the effect of insulin hypoglycemia was investigated.[1] Subsequently, the present study was conducted to explore the effect of bleeding on the noradrenaline and adrenaline contents of the adrenal gland in the cat.

Experimental

Methods

In the present investigation 24 cats were randomized into 3 groups of 4 male and 4 female cats each.

In the first place, control experiments were done in one group of cats (*group 1*) without performing the bleeding. Secondly, blood was taken out from the femoral artery of another group of cats (*group 2*) in an amount of two-fifths of the total blood quantity. Three hours after the bleeding the animals were killed by a blow on the neck and the adrenal glands were extirpated immediately for the estimation of the noradrenaline and adrenaline contents. In *group 3* the first bleeding was done in just the same way as above. One hour after this bleeding blood was taken out again in an amount of one-fifth of the total blood quantity. After 15–50 minutes the animals died without exception. Just after death the adrenal glands were removed.

Each of left and right adrenal gland was extracted with 4% trichloracetic acid. For the differential estimation of the noradrenaline and

adrenaline contents of the extracts the permanganate method of Suzuki and Ozaki[2] was employed.

TABLE I

Effect of Hemorrhage on the Adrenaline and Noradrenaline Contents of the Adrenal Gland in the Cat

No.	Body weight (kg.)	Sex	L or R	Weight of gland (mg.)	Adrenaline and noradrenaline contents (μg.)						Per cent noradrenaline
					per gland		per mg. of gland		per kg. of body weight		
					Adr.	Noradr.	Adr.	Noradr.	Adr.	Noradr.	
Control experiments											
1	2.85	♂	l	205	160	131	0.78	0.64	56	46	45
			r	202	145	121	0.72	0.60	51	42	46
2	3.24	♂	l	218	154	106	0.71	0.49	48	33	41
			r	226	140	115	0.60	0.51	43	35	45
3	2.23	♂	l	109	58	69	0.53	0.63	26	31	54
			r	112	47	60	0.43	0.54	21	27	56
4	2.56	♂	l	165	102	53	0.62	0.32	40	21	34
			r	176	105	46	0.60	0.26	41	18	31
5	2.97	♀	l	134	98	82	0.73	0.61	33	28	46
			r	152	101	93	0.66	0.61	34	31	48
6	2.76	♀	l	228	130	82	0.57	0.36	47	30	39
			r	230	130	77	0.57	0.33	47	28	37
7	2.53	♀	l	332	142	84	0.44	0.26	56	33	37
			r	301	137	80	0.46	0.27	54	32	37
8	2.70	♀	l	298	115	78	0.39	0.26	43	29	40
			r	285	103	87	0.36	0.31	38	32	46
		Mean			*117*	*85*	*0.57*	*0.43*	*42*	*31*	*43*
Bleeding experiments: 2/5 of the total blood quantity											
9	2.54	♂	l	147	53	163	0.36	1.11	21	64	75
			r	149	63	89	0.42	0.60	25	35	59
10	1.74	♂	l	113	38	35	0.34	0.31	22	20	48
			r	118	39	24	0.33	0.20	22	14	38
11	2.88	♂	l	185	48	71	0.26	0.38	17	25	60
			r	182	49	82	0.27	0.45	17	28	63
12	3.38	♂	l	231	78	171	0.34	0.74	23	51	69
			r	245	85	138	0.35	0.56	25	41	62
13	2.42	♀	l	228	169	59	0.74	0.26	70	24	26
			r	244	111	91	0.45	0.37	46	38	45
14	2.70	♀	l	190	77	56	0.41	0.29	29	21	42
			r	196	70	68	0.36	0.35	26	25	49
15	2.48	♀	l	162	52	82	0.33	0.51	21	33	61
			r	158	44	94	0.28	0.59	18	38	68
16	2.42	♀	l	236	69	130	0.29	0.55	29	54	65
			r	233	57	135	0.24	0.58	24	56	70
		Mean			*69*	*93*	*0.36*	*0.49*	*27*	*36*	*53*

Bleeding experiments: 2/5 plus 1/5 of the total blood quantity

17	2.20	♂	l	273	51	43	0.19	0.16	23	20	46
			r	286	44	54	0.15	0.19	20	25	55
18	2.82	♂	l	240	82	72	0.34	0.30	29	26	47
			r	270	57	91	0.21	0.34	20	32	62
19	4.54	♂	l	314	141	182	0.45	0.58	31	40	56
			r	358	92	178	0.26	0.50	20	39	66
20	3.73	♂	l	230	41	50	0.18	0.22	11	13	55
			r	239	43	51	0.18	0.22	12	14	54
21	2.28	♀	l	268	49	50	0.18	0.19	21	22	51
			r	244	38	72	0.16	0.30	17	32	66
22	3.00	♀	l	186	32	56	0.17	0.30	11	19	64
			r	207	31	53	0.15	0.26	10	18	63
23	2.17	♀	l	178	68	74	0.38	0.42	31	34	52
			r	212	84	73	0.40	0.34	39	34	47
24	3.27	♀	l	226	11	41	0.05	0.18	3	13	79
			r	241	14	45	0.06	0.19	4	14	76
		Mean			*55*	*74*	*0.22*	*0.29*	*19*	*25*	*59*

Results

All the data are shown in Table I.

In control experiments the mean values of the adrenal adrenaline and noradrenaline contents were 0.57 μg. and 0.43 μg. per mg. of gland weight, respectively. They were 42 μg. and 31 μg. per kg. of body weight, respectively. The relative amount of noradrenaline was calculated as 43% on the average.

In *group 2*, in which blood was taken out in an amount of two-fifths of the total blood quantity, the mean values of adrenaline and noradrenaline contents of the adrenal gland were estimated to be 0.36 μg. and 0.49 μg. per mg. of gland weight, respectively. They were 27 μg. and 36 μg. per kg. of body weight, respectively. The relative amount of noradrenaline was 53% on the average.

The adrenaline content of the adrenal gland in *group 2* was definitely smaller than that in control experiments. The difference between estimates of both groups was statistically highly significant ($P<0.01$). On the contrary, the adrenal noradrenaline content of *group 2* seemed to be somewhat larger than that of control experiments. The difference was, however, statistically not significant ($P>0,20$).

In *group 3*, the bleedings were carried out twice, i.e. at first in an amount of two-fifths and after one hour one-fifth of the total blood quantity. The mean adrenaline and noradrenaline contents in this group were 0.22 μg. and 0.29 μg. per mg. of gland weight and 19 μg. and 25 μg. per kg. of body weight, respectively. The relative noradrenaline content was 59% in the mean value.

The adrenaline content of the adrenal gland in *group 3* was distinctly

smaller than that in control experiments. The difference was statistically highly significant ($P<0.001$). The adrenal noradrenaline content in this group was found to be somewhat smaller than that in control group. The difference was statistically significant ($P<0.05$).

Discussion

It has been more than three decades since the catecholamine depletion of the adrenal gland causable by hemorrhage was studied and confirmed by several authors.[3] In those studies, however, differential estimation of noradrenaline and adrenaline was not performed.

In the present investigation it was demonstrated that after hemorrhage there was a definite decrease in adrenaline content and no alteration of noradrenaline content of the adrenal gland. After severe hemorrhage, however, there was a slight but definite decrease in noradrenaline content in addition to a marked decrease in adrenaline content of the adrenal gland.

Similar results were obtained in my previous study concerning the effect of insulin hypoglycemia upon the noradrenaline and adrenaline contents of the adrenal gland in the cat.[1] After application of insulin in a dose of 2 units per kg. of body weight a decrease in adrenaline content but no alteration in noradrenaline content of the adrenal gland was found. After 5 units insulin per kg. was given, there were decreases both in adrenaline and noradrenaline contents. However, the former decreased far more markedly than the latter.

Since the glycemic effect of adrenaline is definitely stronger than that of noradrenaline,[4] it seems to be rational that insulin hypoglycemia causes an increase in adrenaline secretion rather than in noradrenaline secretion of the adrenal gland.

As concerns the pressor action of catecholamines, noradrenaline is more active than adrenaline when applied to the cat.[5]–[10] From this viewpoint it is expected that in the case of hemorrhagic hypotension there would chiefly be an increase in noradrenaline secretion and hence there would be noradrenaline depletion of the adrenal gland rather than adrenaline depletion. By the present study, however, this was found not to be the case.

After severe hemorrhage a slight decrease in adrenal noradrenaline content was found in the present study. This does not readily mean the augmentation of the noradrenaline secretion, because in such case there is a possibility of an acceleration of methylation of noradrenaline to adrenaline.

Summary

The present investigation was attempted to know the influence of

bleeding on the adrenal noradrenaline and adrenaline contents of the cat. Noradrenaline and adrenaline contents of the adrenal gland were estimated by the permanganate method of Suzuki and Ozaki.

After the bleeding of two-fifths of the total blood quantity a decrease in adrenaline content of the adrenal gland was found. However, there was no modification of adrenal noradrenaline content.

In cats, in which the bleeding of two-fifths plus one-fifth of the total blood quantity was performed, a marked depletion of adrenaline content and a slight decrease in noradrenaline content of the adrenal gland were found.

The author is deeply indebted to Prof. T. Suzuki for critically reviewing the manuscript and to Dr. M. Motomura for his help in conducting the experiments.

References

1) Ide, M., Kumamoto Med. J., 1959, **12**, 258.
2) Suzuki, T. and Ozaki, T., Tohoku J. Exp. Med., 1951, **54**, 332. Ozaki, T., *ibid.*, 1954, **61**, 83.
3) Tachi, H., *ibid.*, 1928, **10**, 409. (Previous references there)
4) Tanaka, I., *ibid.*, 1956, **63**, 259. (Previous references there)
5) Tainter, M. L., Arch. internat. Pharmacodyn., 1931, **41**, 365.
6) Greer, C. M., Pinkston, J. O., Baxter, Jr. J. H. and Brannon, E. S., J. Pharmacol. Exp. Ther., 1938, **62**, 189.
7) West, G. B., J. Physiol., 1947, **106**, 418.
8) Graham, J. D. P., *ibid.*, 1949, **108**, 15 P.
9) Schümann, H. J., Arch. exp. Path. Pharmakol., 1949, **206**, 164.
10) Takenaka, F., Namba, K. and Hongo, T., Nagasaki Igk. Z., 1952, **27**, 618 (Japanese).

The Tohoku Journal of Experimental Medicine, Vol. 71, No. 4, 1960

Effect of Salt Loading on Renal Hemodynamics and Electrolytes Excretion in Essential Hypertension and in Chronic Glomerulonephritis

By

Chojiro Sasamori
(笹森 長次郎)

From the Department of Internal Medicine, Sendai National Hospital, Sendai; Director: Dr. T. Kato, Professor emeritus of Tohoku University

(Received for publication, November 5, 1959)

In 1905, Ambard and Beaujard[1] found that the administration of NaCl to the patients with hypertension produced a further increase in blood pressure and the restriction of salt resulted in a fall of blood pressure in these patients. Since then, many studies have been attempted to investigate the participation of sodium chloride in the occurrence of hypertension. Dahl[2] investigated the daily salt intake in 1300 subjects and found the high incidence of hypertension in the subjects who took a larger amount of salt. Itahara and others[39] have pointed out, that in the north-eastern district, especially in Akita prefecture, of Japan, where the incidence of hypertension and apoplectic attack is significantly high, compared with that in the other districts, the people use to take up an excess of salt in the daily diet. They are at present making effort to improve the diet, restricting the salt intake, and it is now said that the occurrence of those diseases is decreasing, though slightly, year by year. Sapierstein and his collaborators[3] succeeded in producing a permanent hypertension in rats by administrating hypertonic saline solution every day for a long time. It was reported by Laramore[4] and Eichelberger[5] that the salt content of the muscles was higher in animals with experimental hypertension.

The salt loading test was performed by Markley[6] and others to clarify the mechanism of salt excretion. Some of them[6][7] used the oral way and others[8][9] parenteral way in the test.

The present study was undertaken to investigate the effect of salt loading on the renal hemodynamics and the electrolytes excretion in the patients with essential hypertension and chronic glomerulonephritis with high blood pressure.

Experimental

Method

Test subjects. Test subjects are consisted of 7 normotensive non-edematous adults without any renal and heart diseases, 21 patients with essential hypertension and 15 patients with chronic glomerulonephritis. These subjects were grouped by the blood pressure value, and number of cases, blood pressure ranges and complications were tabulated in Table I.

Table I

Subjects, Ages, Blood Pressure Ranges and Complications

	Group	Cases	Ages	Blood pressure ranges	Complications
Normal	N	7 (M. 3, F. 5)	21–41	106–126/ 65–80	
Essential hypertension (mildly hypertensive)	EM	7 (M. 5, F. 2)	23–66	142–180/ 80–90	a case with diabetes mellitus a case with myocardial damage
„ „ (moderately severely hypertensive)	EMS	9 (M. 7, F. 2)	38–76	164–198/ 90–120	a case with diabetes mellitus 3 cases with myocardial damage 3 cases with hemiplegia
„ „ (severely hypertensive)	ES	5 (M. 2, F. 3)	43–62	200–228/ 102–120	3 cases with myocardial damage
Chronic glomerulo-nephritis (normotensive)	NN	3 (M. 3)	20–50	108–140/ 72–84	a case with pulmonary tuberculosis
„ „ (mildly hypertensive)	NM	5 (M. 3, F. 2)	19–68	140–176/ 80–90	
„ „ (moderately severely hypertensive)	NMS	4 (M. 2, F. 2)	37–40	170–200/ 100–110	a case with myocardial damage a case with hemiplegia
„ „ (severely hypertensive)	NS	3 (F. 3)	32–45	214–250/ 114–147	2 cases with myocardial damage

The 7 control subjects had a diet containing 15 g. of NaCl per day, 8 of the hypertensive group 15 g., 4 cases 8 g., 8 cases 4 g., and one case 2 g. per day. Out of the 15 patients with chronic nephritis, 2 cases had a diet containing 15 g. of NaCl per day, 11 cases 4 g., and 2 cases 2 g. per day. The administration of hypotensive agents was discontinued for 5 days before the test. In the female subjects, the test was made in the post- or intermenstural periods, in order to avoid the abnormality in electrolytes metabolism in premenstural period.

Procedure. After an overnight fast, the test subjects were kept in rest and sodium *p*-aminohippurate (PAH) and mannitol dissolved in 5% glucose solution were infused intravenously to estimate renal plasma flow

and glomerular filtration rate. After 30 minutes of the start of infusion the urine specimens were collected by catheter every 15 minutes prior to and after the saline injection for 45 minutes. The 5% saline solution (2 cc./Kg. of body weight) was injected intravenously within three minutes. The 6 cc. of blood specimen was obtained from the antecubital vein into the heparinized tube every 15 minutes during the test and the plasma was separated by centrifugation. The concentration of PAH and mannitol in the blood and urine specimens were determined using spectrophotometer by method of Page and Corcoran.[10] The determination of Na and K were made flamephotometrically.[11] The content of Cl was estimated by the method of Schales and Schales.[12] Renal plasma flow and others were calculated according to the following formula:

$$\text{Renal plasma flow (RPF)} = \frac{U_{PAH}V}{P_{PAH}}$$

$$\text{Glomerular filtration rate (GFR)} = \frac{U_{man}V}{P_{man}}$$

$$\text{Filtration fraction (FF)} = \frac{GFR}{RPF}$$

$$\text{Renal blood flow (RBF)} = \frac{RPF}{(100-Ht)} \times 100$$

P_{PAH} : PAH concentration in plasma
U_{PAH} : PAH concentration in urine
P_{man} : Mannitol concentration in plasma
U_{man} : Mannitol concentration in urine
V : Minute urine volume (cc./min.)

The injection of the hypertonic saline solution sometimes provoked a heat sensation of the body and thirsty sensation in the mouth which disappeared usually within a few minutes.

Results

I. Essential hypertension

1. *Effect on renal hemodynamics*

The RPF at the pre-load period decreased with an increase in blood pressure and this value was decreased in EM on an average by 27.7% of that of the normal group, in EMS by 30.2% and ES by 41.5%, respectively (Table II–2). The similar relationships were observed in RBF (Table II–1) and GFR (Table II–3). The decrease rates of GFR in the groups of EM, EMS and ES in comparison with the normal group were 13.9%, 19.0 % and 31.4%, respectively (Table II–3). The FF value was usually higher in the hypertensive groups, than in the normal group (Table II–4).

In the normal group the saline injection produced a slight depression in RPF at the first period, which rebounded to the pre-load level at the second

TABLE

Renal Hemodynamics and Electrolytes Excretion before

Group			Normal		Essential
			N		EM (mildly
Cases			7		7
MABP mm. Hg			79–92		101–
Period			0	I–III	0
1	RBF cc/min.	Mean	(747–1500) 1128.8	(781–1613) 1125.3 −0.3%	(496–1151) 836.5
2	RPF cc/min.	"	(430–893) 644.1	(460–814) 640.3 −0.6%	(305–647) 464.7
3	GFR cc/min.	"	(81–130) 95.2	(80–119) 95.4 +0.2%	(62–114) 82.0
4	FF	"	(0.112–0.198) 0.154	(0.126–0.183) 0.152	(0.096–0.267) 0.186
5	Urine V. cc/min.	"	1.40–7.07) 4.010	(1.69–3.11) 2.367 −41.1%	(0.87–6.35) 3.443
6	Na Exc. μ Eq/min.	"	(120–560) 289.0	(116–530) 283.3 −1.8%	(92–530) 273.7
7	Cl Exc. μ Eq/min.	"	(104–380) 219.6	(147–427) 246.0 +1.2%	(46–460) 235.3
8	K Exc. μ Eq/min.	"	(47–150) 76.4	(38–57) 43.1 −43.6%	(25–58) 39.4

O: Before load

I–III: After load (average value of I–III period)

load period. In the hypertensive groups, the saline load resulted in an elevation of RPF at the first load period followed by a decrease from the second period. This decrease in RPF was remarkable in EMS and ES (Fig. 1–a). A similar tendency was observed in RBF and GFR (Fig. 1–b). The FF did not change in the normal group even after the saline injection, but it increased in the hypertensive groups.

2. *Effect on plasma electrolytes*

The plasma Na level at the pre-load period was higher on an average in the hypertensive groups than the normal group with an exceptions of

II

and after Saline Load in Normal and Essential Hypertension

hypertensian				
hypertensive)	EMS (moderately severely hypertensive)		ES (severely hypertensive)	
	9		5	
120	122–140		144–150	
I–III	0	I–III	0	I–III
(477–1045) 833.8 −0.3%	(419–1183) 773.6	(409–1029) 751.3 −2.8%	(279–921) 651.8	(284–866) 626.3 −3.9%
(332–607) 479.5 +1.1%	(242–651) 448.9	(242–556) 437.8 −2.5%	(162–476) 376.2	(168–465) 357.8 −4.9%
(66–122) 87.6 +6.5%	(59–102) 77.2	(58–122) 81.3 +5.3%	(15–112) 65.4	(18–102) 67.2 +3.4%
(0.109–0.225) 0.188	(0.100–0.293) 0.183	(0.104–0.285) 0.197	(0.093–0.235) 0.161	(0.107–0.219) 0.177
(0.95–5.95) 2.720 −20.9%	(0.67–8.27) 3.671	(0.71–5.87) 3.092 −15.7%	(0.67–5.93) 3.240	(1.00–6.18) 3.788 +16.9%
(106–744) 358.4 +27.2%	(110–760) (380.0)	(139–1141) 564.0 +48.5%	(44–681) 368.6	(131–977) 539.8 +46.6%
(82–655) 316.4 +34.5%	(72–536) 289.2	(83–856) 379.2 +31.3%	(20–590) 289.8	(107–754) 440.4 +55.2%
(24–61) 42.1 +8.4%	(25–105) 64.8	(21–121) 64.7 −0.2%	(33–60) 41.6	(33–80) 59.6 +44.3%

MABP: Mean arterial blood pressure, calculated as the third pulse pressure plus diastolic pressure.

EMS (Fig. 2–a). However, there was no clear relationship between the grade of hypertension and the Na, Cl and K levels.

The Na level became maximum at the first period after saline injection and the increase rate at this period compared with the pre-load level was 2.2% for N, 2.3% for EM, 1.8% for EMS and 4.2% for ES. A similar relationship was observed in the Cl level (Fig. 2–b). There was no relationship between the grade of hypertension and the increase of Na and Cl concentrations. The plasma K level was lowered by the saline injection at the first or second load period and the maximum decrease rate

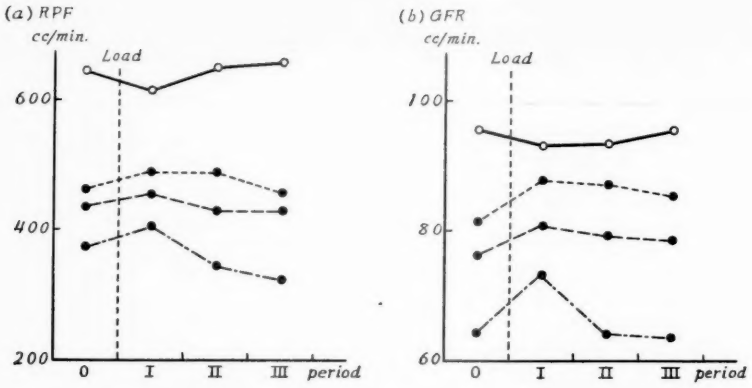

Fig. 1. Hemodynamic Changes after Hypertonic Saline Load in Essential Hypertension (mean value of RPF and GFR).
○——— Group of Normal Subjects
●- - - - ,, ,, Essential Hypertension (mildly hypertensive)
●—— ,, ,, ,, ,, (moderately severely hypertensive)
●—·— ,, ,, ,, ,, (severely hypertensive)

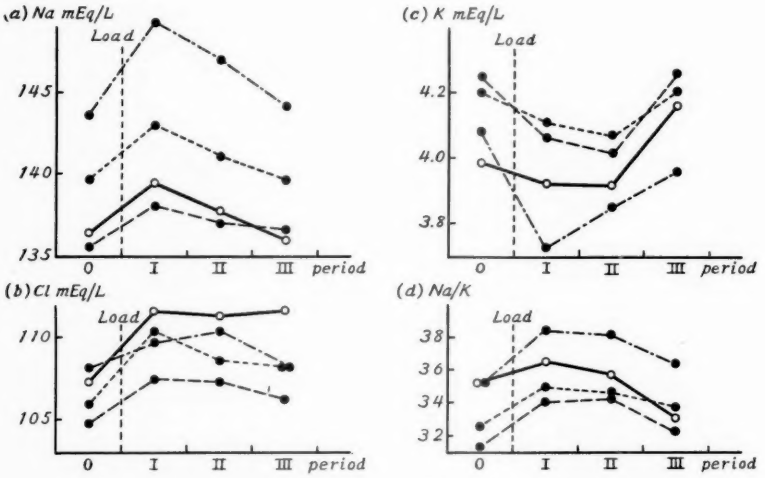

Fig. 2. Plasma Electrolytes Concentration after Hypertonic Saline Load in Essential Hypertension.
○——— Group of Normal Subjects
●- - - - ,, ,, Essential Hypertension (mildly hypertensive)
●—— ,, ,, ,, ,, (moderately severely hypertensive)
●—·— ,, ,, ,, ,, (severely hypertensive)

was 1.8% for N, 3.1% for EM, 5.9% for EMS and 8.8% for ES (Fig. 2–c). The Na/K ratio was elevated by the saline injection and this increase rate was 3.7% for N, 4.9% for EM, 8.2% for EMS and 11.0% for ES, respectively (Fig. 2–d).

3. *Effect on minute urine volume*

There was no regular relationship between the grade of hypertension and urine volume in pre-load period. In the normal group, urine volume decreased after the saline injection and in hypertensive groups, this inhibiting effect of saline injection on diuresis was not remarkable, especially in ES (Fig. 3–a). The decrease rate of mean volume in 3 periods compared with the pre-load period, was −41.1% for N, −20.9% for EM, −15.7% for EMS and +16.9% for ES, respectively (Table II–5).

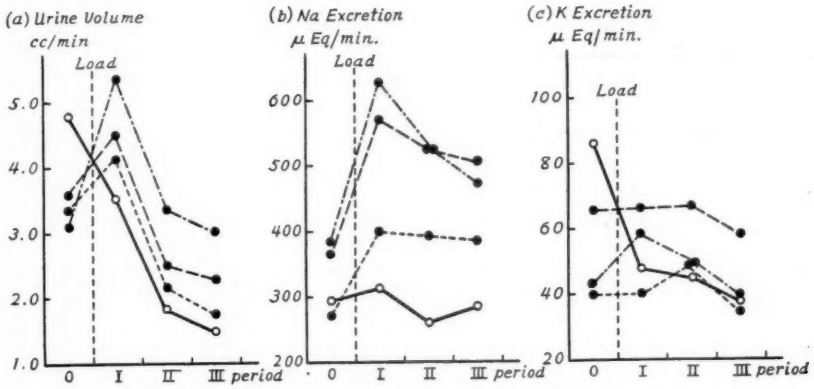

Fig. 3. Water and Electrolytes Excretion after Saline Load in Essential Hypertension.

○—— Group of Normal Subjects
●- - - - „ „ Essential Hypertension (mildly hypertensive)
●—— „ „ „ „ (moderately severely hypertensive)
●—— „ „ „ „ (severely hypertensive)

4. *Effect on minute electrolytes excretion*

Any relationship was not observed between the severity of hypertension and the electrolytes excretion at the pre-load period (Table II–6, 7, 8).

After the saline injection the urinary excretion of Na rose during the first period and thereafter it fell (Fig. 3–b). The mean ratio of the excreted Na before and after the saline load was −1.8% for N, +27.2% for EM, +48.5% for EMS and +46.6% for ES (Table II–b). The excretion of Na became greater with an increase in blood pressure. A similar phenomenon was seen in the excretion of Cl. The excretion of K

TABLE

Renal Hemodymanics and Electrolytes Excretion before and after Saline

Group			Chronic		
			NN (normotensive)		NM (mildly
Cases			3		5
MABP mm Hg			87-95		102-
Period			0	I-III	0
1	RBF cc/min.	Mean	(236-975) 672.0	(270-1089) 682.7 +1.6%	(32-965) 536.8
2	RPF cc/min.	"	(111-517) 357.7	(142-592) 385.7 +7.9%	(27-707) 342.0
3	GFR cc/min.	"	(9-122) 61.0	(10-119) 61.3 +0.5%	(14-62) 44.0
4	FF	"	(0.081-0.236) 0.144	(0.071-0.201) 0.132	(0.088-0.520) 0.216
5	Urine V. cc./min.	"	(0.67-1.30) 0.960	(0.78-1.07) 1.017 +6.0%	(1.00-1.67) 1.456
6	Na Ex. μ Eq/min.	"	(40-210) 118.0	(73-237) 137.0 +16.1%	(40-200) 131.0
7	Cl Exc. μ Eq/min.	"	(5-150) 79.0	(28-173) 87.0 +10.0%	(59-157) 98.6
8	K Exc. μ Eq/min.	"	(17-130) 68.7	(22-105) 56.0 -18.7%	(17-185) 73.2

O: Before load

was decreased by the saline injection in the normal group but it was elevated or unchanged in the hypertensive groups (Fig. 3-c).

II. Chronic glomerulonephritis

1. *Effect on renal hemodynamics*

The mean RPF of the nephritis group was less than that of normal group and it was lower in the group of chronic nephritis with hypertension than in that without hypertension (Table III-2). It became smaller with an increase in blood pressure. The GFR was lower in the chronic ne-

III

Load in Chronic Glomerulonephritis with and without Hypertension

glomerulonephritis				
hypertensive)	NMS (moderately severely hypertensive)		NS (severely hypertensive)	
	4		3	
118	125-130		147-177	
I-III	0	I-III	0	I-III
(33-1006) 550.2 +2.5%	(300-670) 499.3	(278-785) 532.2 +6.6%	(22-957) 439.3	(18-1037) 463.7 +7.8%
(27-640) 335.6 −2.2%	(152-390) 298.5	(152-456) 308.5 +3.3%	(18-475) 236.7	(14-499) 245.7 +3.8%
(14-69) 45.8 +4.1%	(48-118) 75.2	(50-107) 79.5 +5.7%	(9-90) 53.0	(8-92) 53.3 +0.6%
(0.090-0.569) 0.231	(0.221-0.313) 0.265	(0.211-0.330) 0.265	(0.217-.529) 0.342	(0.183-0.571) 0.341
(1.08-2.20) 1.558 +7.0%	(1.07-2.34) 1.485	(2.00-4.61) 3.158 +112.3%	(0.60-8.32) 4.572	(0.78-8.17) 5.03 +9.9%
(83-290) 168.8 +28.8%	(85-264) 137.5	(139-545) 335.0 +144.5%	(45-526) 290.3	(62-865) 533.3 +83.9%
(75-266) 159.8 +61.9%	(22-190) 90.5	(118-479) 310.0 +244.3	(58-720) 376.0	(79-1098) 581.3 +57.2
(15-224) 72.0 −1.6%	(28-121) 64.0	(52-146) 78.5 +22.6%	(14-96) 62.3	(15-119) 60.7 −2.6%

I-III: After load (average value of I-III period)

phritis groups than in the normal and essential hypertension groups of same blood pressure range (Table III-3). The FF was higher in these groups than in normal and essential hypertension (Table III-4).

The changes of RPF after the saline injection are depicted in Fig. 4-a. There was no definite relationship between the increase rate of RPF after saline load and severity of hypertension (Table III-2). A similar change was also observed in RBF (Table III-1). The fluctuation of GFR after the saline injection is charted in Fig. 4-b, and the changes of FF are summerized in Table III-4.

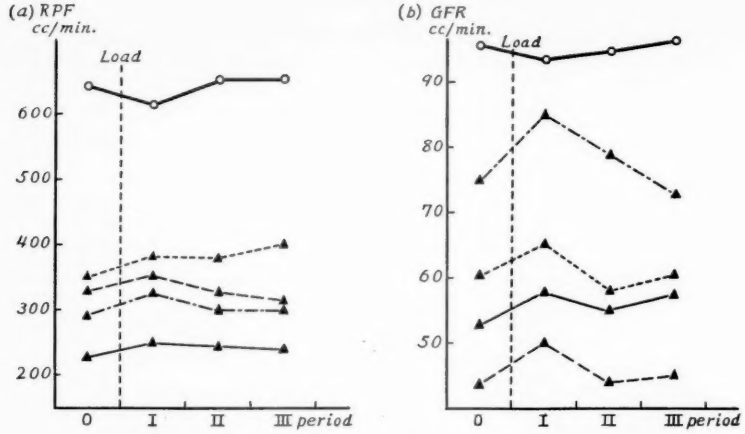

Fig. 4. Hemodynamic Changes after Saline Load in Chronic Glomerulonephritis with and without Hypertension (mean value of RPF and GFR).

○——— Group of Normal Subjects
▲- - - - „ „ Chronic Glomerulonephritis (normotensive)
▲— — — „ „ „ „ (mildly hypertensive)
▲— - — „ „ „ „ (moderately severely hypertensive)
▲——— „ „ „ „ (severely hypertensive)

2. *Effect on plasma electrolytes*

The plasma Na concentration at the pre-load period was higher in the groups of chronic nephritis than in the normal group and this value has an inclination to become larger with an increase in blood pressure (Fig. 5–a). A similar relation was observed in the plasma K concentration (Fig. 5–c), but not in plasma Cl level (Fig. 5–b).

The saline injection provoked the increases in plasma Na, Cl and Na/K ratio and the decrease in plasma K (Fig. 5). The changes in plasma K and Na/K were more prominent in the groups with high blood pressure.

3. *Effect on minute urine volume*

The urine volume at the pre-load period was lower in this group than in the normal and the groups with essential hypertension. The changes after the saline load are charted in Fig. 6–a.

4. *Effect on minute electrolytes excretion*

The electrolytes excretion of Na and Cl at the pre-load period were less in this group than in the normal group and the groups with essential hypertension (Fig. 6–b).

The saline injection caused an increase of Na excretion in the all groups of chronic nephritis and the increase of Na excretion after load

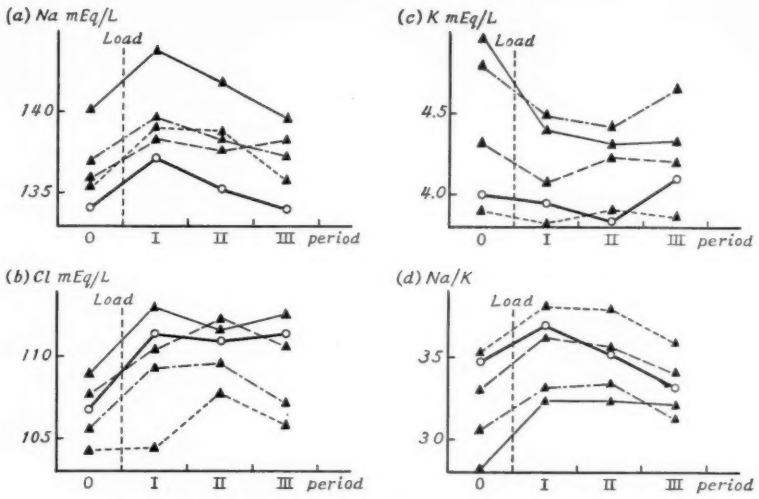

Fig. 5. Plasma Electrolytes Concentration after Saline Load in Chronic Glomerulonephritis with and without Hypertension (mean value of each group).

○—— Group of Normal Subjects
▲- - - - „ „ Chronic Glomerulonephritis (normotensive)
▲— — „ „ „ „ (mildly hypertensive)
▲— — „ „ „ „ (moderately severely hypertensive)
▲—— „ „ „ „ (severely hypertensive)

was +16.1% for NN, +28.8% for NM, +144.5% for NMS and +83.9% for NS (Table III–6). These rates in the nephritis group were significantly greater than those in the normal group and this rate became more augmented with an increase in blood pressure. The chnges of electrolytes excretion of K are shown in Fig. 6–c.

Fig. 7 shows the relationship between the mean arterial blood pressure and the increased electrolytes excretion of Na in each of the test subjects. A linear relationship is seen between them. The relation between GFR and the electrolytes excretion of Na is charted in Fig. 8. A linear relationship is also demonstrated.

The effect of saline injection the on blood pressure is summerized in Table IV. It is hard, however, to find a definite trend of changes in it.

The typical cases were charted in Fig. 9.

To summerize, the cases with hypertension, in the groups of essential hypertension as well as in the groups of chronic nephritis, showed an excretion of larger amount of Na and Cl than the cases without hypertension.

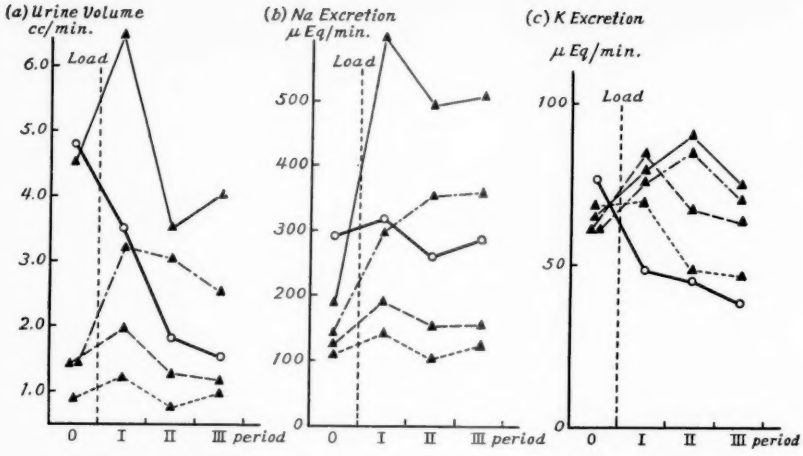

Fig. 6. Water and Electrolytes Excretion after Saline Load in Chronic Glomerulonephritis with and without Hypertension.

○—— Group of Normal Subjects
▲- - - - „ „ Chronic Glomerulonephritis (normotensive)
▲— — — „ „ „ „ (mildly hypertensive)
▲— — — „ „ „ „ (moderately severely hypertensive)
▲—— „ „ „ „ (severely hypertensive)

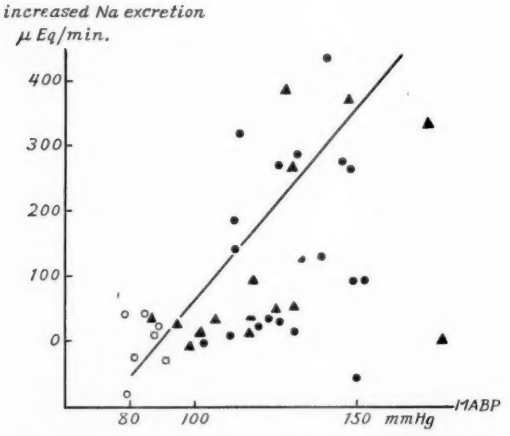

Fig. 7. Correlation between Mean Arterial Blood Pressure and Increased Sodium Excretion.

○ Normal ● Essential Hypertension ▲ Chronic Glomerulonephritis with and without Hypertension

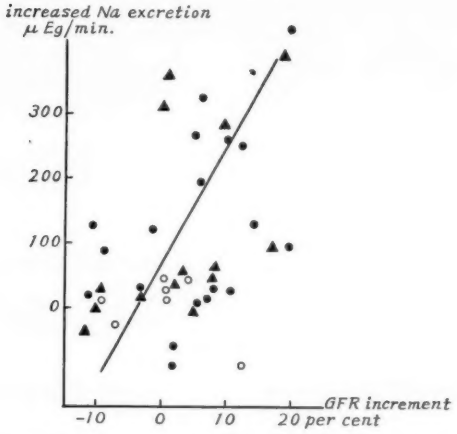

Fig. 8. Correlation between Increased Sodium Excretion and GFR Increments after Load.

○ Normal ● Essential Hypertension ▲ Chronic Glomerulonephritis with and without Hypertension

TABLE IV

Mean Arterial Blood Pressure after Saline Load

(average value in each group)

Period	Before load 0	After load I	II	III
Group N	84.2	84.2	82.0	84.8
" EM	110.0	109.7	108.0	106.7
" EMS	132.0	130.6	127.8	125.6
" ES	145.8	144.4	137.6	135.6
" NN	91.0	89.0	88.0	86.0
" NM	115.0	114.7	116.0	114.0
" NMS	128.3	127.6	125.0	130.3
" NS	158.3	162.6	161.6	159.3

Discussion

Green[8] performed an intravenous saline loading test in 79 subjects with and without hypertension and he found that the urinary excretion of Na before and after the saline injection was significantly greater in the subjects with hypertension. He emphasized that the patients with hypertension could be classified into the " normal salt-excretor " and the " high salt-excretor ". In the " high salt-excretor ", by his definition, the urinary

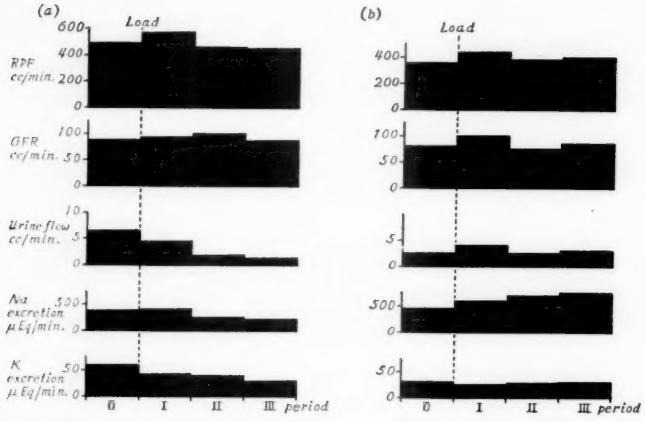

Fig. 9. Representative Examples. (a) Normal: A. K. 33 F. (B. P. 110/62) (b) Essential Hypertension: S. M. 62 M. (B. P. 210/120)

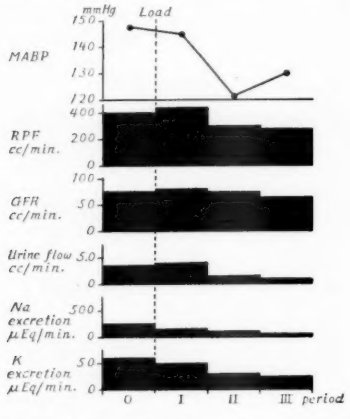

Fig. 10. An Example Revealing Blood Pressure Depression after Load. Essential Hypertension: O. S. 43 F. (B. P. 204/120)

excretion of salt was elevated in the basal state as well as in the saline loading period, and an increased appetite for salt, a normal GFR, a normal cardiac output and elevation of FF were observed as the result of the decrease in RPF. While, the " normal salt-excretor " was characterized by a normal salt excretion, a normal appetite for salt and a normal FF, which was the result of the decrease in GFR and RPF. He stated further that the " high salt-excretor " corresponded with the early stage of hypertension and the " normal salt-excretor " with the later stage of hyper-

tension and that the increase of salt excretion after saline load might be an augmentation of the high salt excretion at the basal state. A similar observation was obtained by Cottier.[13)14)] Hollander and his co-worker[15)] injected 300 cc. of 2.5% saline solution to the patients with essential hypertension and found an increase in urinary excretion of salt for 25–30 minutes after the saline injection. They observed a normalization of " high salt-excretion " by the long term administration of hypotensive agents, and speculated that the hypertension might influence directly on the renal tubular function, and that arterial pressure may play an important role in the control of sodium excretion.

Farnsworth and his co-workers[16)–18)] observed the higher excretion of Na and Cl after the saline injection in the patients with essential hypertension in comparison with normal subjects. Brodsky and his co-workers[19)] also observed that the urinary excretion of Na and Cl were $2-2\frac{1}{2}$ times in 6 patiens with hypertension as high as in normal subjects. Both of these workers supposed the presence of impaired reabsorption mechanism of water and Na in the renal tubules of the hypertensive patients.

Birchall and his co-workers[20)] injected 2.5% NaCl solution (0.25 cc./Kg. of body weight /min.) for 45 minutes and observed the urinary excretion of NaCl for 45 minutes after the end of the saline injection and found that the hypertonic saline injection inhibited the water and salt excretion in the normal subjects but this inhibiting effect was less in the patients with hypertension. This inhibiting effect of the saline injection became decreased with an increase in blood pressure. They supposed that the cause of the high salt and water excretion in the patients with hypertension might be due to a lowered secretion of antidiuretic hormon by the stimulation of saline injection, *i.e.*, the hypertensive patients showed some impaired function in the hypothalamico-neuro-hypophyseal system.

The results of the other workers[21)–25)] on saline load experiments agree with the results of the present experiments. The results of the present study, in which the salt excretion of the hypertensive patients at the basal period is slightly increased and that after the saline load is remarkably increased in comparison with the normotensive subjects, are coincident with the results of Green[8)] and Cottier.[13)]

These experimental results seem to indicate the influence of blood pressure on the excretion of salt and water. Selkurt and his co-workers[26)] made an artificial stenosis of abdominal aorta just above the branching of right renal artery in 5 dogs and found that the lowering of blood pressure in the right renal artery caused a decrease in Na excretion and in GFR without any change in RBF. Richards[27)] found that the elevation of blood pressure without any change in RBF provoked the increases in urine volume and the urinary excretion of electrolytes in rabbits.

Verney[28)29)] found that the ligation of some branch of renal artery caused the increase in water and Cl excretion and they thought these increases might be due to an increase in renal arterial blood pressure in this side. Shipley[30)] observed an increase in urine volume when he raised the renal blood pressure in dogs and he concluded that the elevation of blood pressure increased the tissue pressure in the kidney which might change the tubular rejection of water and electrolytes. A similar effect was also observed by compressing the capsula of the kidney. These experiments demonstrate that the elevation of blood pressure promotes the excretion of the salt and water. Fig. 10 shows a case, in which the excretion of Na and the urine volume decreased with the fall of blood pressure. This case also indicates an important role of blood pressure in the excretion of Na and water.

The next important factor, influencing on the excretion of Na, is the hemodynamics of kidney. The decreases in RPF and GFR reduce a urine volume and the renal excretion of electrolytes.[26)31)] In present study, the excretion of water and sodium was lower in normotensive nephritis group than in normal subjects, and similar trend was observed in hypertensive nephritis groups compared in the same blood pressure range with groups of essential hypertension. It may be the cause of this lowered excretion of Na and water in nephritis group, that RBF and GFR were decreased in these groups than in normal or essential hypertension groups of the same blood pressure range. Merill[32)] and Mokotoff[33)] reported that the decreased cardiac output in heart failure provoked the decrease of RPF and GFR, and that the decrease in GFR reduced the Na excretion and caused an edema. These facts suggest that an increase in the Na excretion is possibly due to an increased GFR and it is demonstrated in Fig. 7 that a linear relationship is presented between GFR and Na excretion. Therefore, one of the causes of the greater excretion of Na in the hypertensive patients may be an increase in GFR after the saline load in these patients, although another possible cause is an indirect effect of hypertension on tubular absorption of Na and water, as pointed out by Farnsworth[16-18)] and Shipley.[30)] Cottier[13)] and Baldwin *et al.*[23)] faild to find any effect of the salt load on GFR, but Hollander *et al.*[15)] found an increase in GFR after the salt load in hypertensive patients, although no change in the normal subjects.

It was reported that the concentration of plasma Na and Cl also influenced on the renal excretion of Na and Cl, as Barger *et al.*[34)] injected the saline solution into a unilateral renal artery and found the greater excretion of NaCl from the injected side. The present experiment, however, failed to show any relationship between the renal excretion of NaCl and the plasma Na level after the saline injection. It was reported by Cottier *et al.*[13)] that there was no difference in an increment of plasma Na

induced by the saline injection between the normal subjects and the patients with hypertension.

In the present experiment, where the test subjects were grouped by the grade of hypertension, showed that the RPF and GFR were the least in the group of severely hypertensive patients. Further, it was found in the present study that the excretion of Na became greater with an advance of hypertension as far as the renal function was normal. It seems, as indicated by Cottier,[13] that the " high salt-excretor " corresponds with the stage where the renal function is not impaired and the " normal salt-excretor " with the stage where the renal function is damaged.

Setooka[35] reported that the excretion of Na after the saline load was less in the patients with essential hypertension or chronic nephritis than in the normal adults. It is one of the causes of this discrepancy between his result and the present result, that his test subjects contained the cases with edema.

Larmore *et al.*[4] found the increased Na content in the muscles, brain and liver of the rabbits with experimental hypertension. Tobian[36] found the increased Na and water contents and decreased K content in the arterial vessel wall in the rats with experimental hypertension.

These facts indicates the sodium retention in the body of the patients with hypertension. It was suggested by Birchall *et al.*[20], that the patients with hypertension would have a surfeit of tissue sodium and chloride, and therefore reject additional salt more rapidily. We may consider that the retention of Na in the patients with hypertension is not due to the impairment of Na excretion from the kidny but due to other factors, for example, abnormal secretion of hormons regulating electrolytes.

The mechanism of the depressor effect of Na-restricted diet is not yet clear. Friedman *et al.*[37] found that the fluctuation of blood pressure provoked a movement of Na through the cell membran, *i.e.*, an elevation of blood pressure accelerated the entrance of Na and water into cells. Dustan *et al.*[38] found that the depression of blood pressure by Chlorothiazide caused an increase of urinary Na excretion and decrease in plasma Na concentration and they speculated that the depressor effect of Chlorothiazide presumeably depends on the same mechanism in low salt diet therapy. The author feels that the mechanism of the curative effect of Na-restricted diet on hypertension may be found in the reduction of intracellar Na concentration—that reduction produced by the emigration of NaCl from the cells into the extracellar fluid which occurs in order to supply or shift the decreased concentration of this fluid due to the restriction of Na intake.

Therefore, it is necessary for the patients with essential hypertension or with chronic nephritis accompanied by hypertension to keep the low-

salt diet even after the normalizing of their elevated blood pressure, because a high salt diet is apt to produce a retention of Na in the body in such a recovery condition.

Summary

The salt loading test was performed in 7 normal aduls, 21 patiens with essential hypertension and 15 patients with chronic nephritis with and without hypertension. After an overnight fast, 5% saline solution was injected intravenously every 2 cc. per Kg. of body weight and the changes in plasma electrolytes, renal hemodynamics, urine volume and urinary excretion of electrolytes were observed.

The saline injection provoked the increase in plasma Na and Cl concentrations, and the decrease in plasma K concentration was more prominent in the groups of essential hypertension and chronic nephritis with hypertension and it became greater with an increase in blood pressure.

A linear relationship was observed between an increase in urinary excretion of Na and increase in glomerular fitration rate which were produced by the saline injection. In the patients with essential hypertension and with chronic hypertensive glomerulonephritis, the urinary excretion of Na after the saline injection became greater with an increase in blood pressure.

The present experimental results seem to indicate that hypertension promotes a urinary excretion of Na, unless any remarkable decrease in renal blood flow and glomerular filtration rate exists.

The author wishes to express his thanks to Dr. F. Nakazawa, Professor emeritus of Tohoku University, and to Dr. H. Odashima, for their advice in consultation.

References

1) Fishberg, Hypertension and Nephritis, Philadelphia, 1954, 724.
2) Dahl, J. Am. Med. Assoc., 1957, **164**, 397.
3) Sapierstein *et al.*, Proc. Soc. Exp. Biol. & Med., 1950, **73**, 82.
4) Laramore *et al.*, Am. J. Phisiol., 1950, **161**, 278.
5) Eichelberger, J. Exp. Med., 1943, **77**, 205.
6) Markley *et al.*, J. Clin. Invest., 1957, **36**, 303.
7) Despoulos *et al.*, J. Clin. Endoclinol., 1952, **12**, 955.
8) Green *et al.*, Circulation, 1954, **9**, 416.
9) Hollander *et al.*, J. Clin. Invest., 1957, **36**, 1460.
10) Page & Corcoran, Arterial Hypertension, 2 ed., Chicago, 1949.
11) Severinghaus *et al.*, J. Biol. Chemi., 1950, **189**, 621.
12) Schales & Schales, *ibid.*, 1941, **140**, 879.
13) Cottier *et al.*, Circulation, 1958, **17**, 750.
14) Cottier *et al.*, *ibid.*, 1958, **18**, 197.
15) Hollander *et al.*, *ibid.*, 1958, **18**, 576.

16) Farnsworth *et al.*, Proc. Soc. Exp. Biol. & Med., 1943, **52**, 74.
17) Farnsworth *et al.*, *ibid.*, 1943, **53**, 160.
18) Farnsworth *et al.*, J. Clin. Invest., 1946, **25**, 897.
19) Brodsky *et al.*, J. Lab. & Clin. Med., 1953, **41**, 43.
20) Birchall *et al.*, Circulation, 1953, **7**, 258.
21) Green *et al.*, *ibid.*, 1952, **6**, 919.
22) Taquini *et al.*, Acta cardiol., 1956, **11**, 109.
23) Baldwin *et al.*, Am. J. Med., 1958, **28**, 893.
24) Ullman *et al.*, Circulation, 1959, **19**, 729.
25) Weller *et al.*, Ann. Int. Med., 1959, **50**, 106.
26) Selkurt *et al.*, Am. J. Physiol., 1949, **159**, 369.
27) Richards *et al.*, *ibid.*, 1922, **59**, 144.
28) Verney, Lancet, 1929, **216**, 539.
29) Verney, *ibid.*, 1930, **219**, 63.
30) Shipley *et al.*, Am. J. Physiol., 1951, **167**, 676.
31) Berliner, Am. J. Med., 1950, **9**, 541.
32) Merill, J. Clin. Invest., 1946, **25**, 389.
33) Mokotoff, *ibid.*, 1948, **27**, 1.
34) Barger *et al.*, Fed. Proc., 1956, **15**, 8.
35) Setooka *et al.*, Saishin Igaku (Jap.), 1956, **11**, 2535.
36) Tobian, J. Exp. Med., 1943, **77**, 205.
37) Friedman *et al.*, Circulation, 1956, **14**, 938.
38) Dustan *et al.*, *ibid.*, 1959, **19**, 360.
39) Itahara *et al.*, Akita Ishikai Zasshi (Jap.), 1952, **4**, 14.

The Tohoku Journal of Experimental Medicine, Vol. 71, No. 4, 1960

Direct Responses to Hippocampal Stimulation in the Rabbit

By

Hisao Suzuki
(鈴木 寿夫)

From the Physiological Laboratory of Prof. K. Motokawa, Tohoku University, Sendai

(Received for publication, November 10, 1959)

Introduction

In the previous report,[1] it was found that the form of the direct response of the neocortex was dependent upon the depth into which the recording electrode was inserted. However, the structure of the neocortex is so complicated that it is very difficult to clarify more detailed correlation between the response and the structure. In comparison with the neocortex, the hippocampus has rather simple laminar structure as the pyramidal cells are confined within a single dense band. Therefore, it seems profitable to make similar experiments on the hippocampus. Cragg and Hamlyn[2] investigated the evoked potential of the hippocampus to direct stimulation, but their interest was directed mainly to the conduction mechanism of the dendrite of the hippocampal pyramid, so that no detailed investigation was made on the properties of the response. In the present experiment, the response was classified according to the layers of the hippocampal stimulation and the properties of each type of response were investigated.

Experimental

Method

All experiments were done on rabbits. Under pentobarbitone anesthesia (30 mg./kg.), the trachea was cannulated and the dorsal surface of the cerebral cortex was exposed as widely as possible in the routine procedure. Then, the neocortex and the underlying white matter were gently aspirated by a vacuum pump until the surface of the dorsal hippocampus appeared. Bleeding was controlled with oxidized cellulose. After the operation was finished, the animal was allowed to recover from the anesthesia, succinylcholine chloride was administered intravenously to immobilize the animal, and the artificial respiration was begun. Occasionally, succinylcholine chloride was supplemented as necessary. Throughout

the experiment, the brain was kept warm with a heat radiator.

The stimulating electrode used consisted of a silver wire 20–30 μ in diameter insulated by a glass capillary to the tip. The electrode was inserted vertically from the ependymal surface of the hippocampus in order to carry out laminar stimulation of it. As a stimulus, a negative square pulse of 0.05 msec. duration or an exponentially decayed pulse with a time constant of 0.07 msec. was used.

Monopolar recording of the response was done at the ependymal surface by means of a silver wire electrode insulated except the tip. The electrode was arranged 1–2 mm. away from the site of insertion of the stimulating electrode. The record through this surface lead served as a control. In each experiment, intralaminar records were taken with a microelectrode in the depth immediately beneath the surface electrode mentioned above. The microelectrode was finely pointed steel needle measuring about 10 μ at the uninsulated tip. The reference electrode was placed over the frontal sinus.

R-C amplifiers with a time constant of 0.03–0.5 sec. and a cathode-ray oscilloscope of two beams were used.

The arrangement of the stimulating and recording electrodes is illustrated schematically on the left side of Fig. 1. On the right side, the laminar structure of the hippocampus is shown.

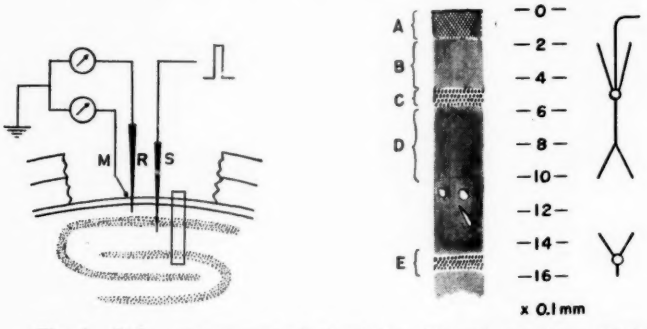

Fig. 1. Left: arrangement of stimulating (S) and recording (R,M) electrodes. Right: schematic representation of laminar structure of hippocampus (part outlined by rectangle in left diagram). A: alveus. B: layer of basal dendrites of hippocampal pyramids. C: layer of somata of pyramids. D: layer of apical dendrites. E: layer of granule cells of dentate gyrus. Numerals indicate depth beneath ependymal surface.

Results

Laminar stimulation of the hippocampus

As the stimulating electrode was inserted vertically into the hippo-

campus to stimulate various depths, recording of the response was made both at the ependymal surface and at a depth 0.6 mm. beneath the surface. The distance between the surface electrode and the site of insertion of the stimulating electrode was about 1 mm. The results are shown in Fig. 2.

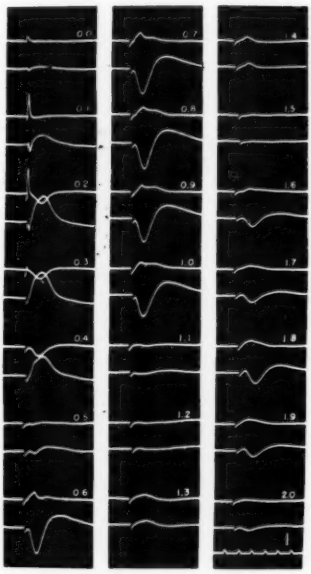

Fig. 2. Records at surface (lower line of each pair) and at depth (upper line), stimulation being performed at depth indicated in mm. on each record. Time signal: 100/sec. Vertical bar: 1 mV. Negativity upward.

In this figure, the upper beam of each pair of records refers to the depth electrode, and the lower one to the surface electrode. Numerals show the depth in mm. where the stimulation was done. The stimulus current was about 100 μA.

The stimulus applied at a depth 0–0.05 mm. beneath the ependymal surface evoked a small negative spike potential of about 1 msec. duration recorded at the surface, and a large negative spike potential of 2–3 msec. duration recorded at the depth. As the stimulating electrode was screwed down and arrived at a depth 0.1–0.2 mm. beneath the ependyma, the spike potentials of the surface and depth were followed by a wave. This wave was a negative one of about 20 msec. in duration recorded at the surface and a positive one at the depth corresponding in time with the surface response. On advancing the stimulating electrode into the deeper layer, the spike potentials recorded at the surface and depth were reduced in

amplitude gradually and finally disappeared when the stimulating electrode arrived at a depth 0.3 mm. beneath the ependyma. With stimulation at a level from 0.3 mm. to 0.4 mm. beneath the ependyma, only the slow waves were observed. Disappearance of the waves occurred when the stimulating electrode arrived at a depth 0.5 mm. beneath the ependyma. With further insertion of the stimulating electrode beyond a depth 0.6 mm. beneath the ependyma, another slow wave appeared, and was maintained till a depth 1.0 mm. beneath the ependyma. The wave was a positive one of 15–20 msec. duration recorded at the surface, but a negative one of 15–20 msec. duration at the depth. When the stimulus strength was high or the animal was in a good physiological state, the peak of the negative wave recorded at the depth was superimposed by a spike-like potential of negative deflection of about 3–5 msec. duration. When the stimulating electrode was inserted further, slow potentials of various patterns were observed, but these potentials varied so widely from experiment to experiment, that it was difficult to classify them into certain types. Within the depth of 1 mm. it was, however, very easy to distinguish three types of response, and it is to be noted that this depth corresponds to the level where the apical dendrite of the hippocampal pyramid terminates. The types are as follows: (I) The spike potential evoked by stimulation at a level 0–0.2 mm. beneath the ependyma (SP), (II) the slow wave evoked by stimulation at a level 0.2–0.4 mm. beneath the ependyma (SW), and (III) the slow wave with a spike like potential superimposed obtainable by stimulation at a level 0.6–1.0 mm. beneath the ependyma.

Laminar recording of the hippocampus

In order to get further information about the laminar distribution of the three different types of response, the stimulating electrode was fixed at a certain level, and the recording microelectrode was inserted into various depths. An example of the experiments is shown in Fig. 3.

In experiment A and A′, stimulation was performed at a depth 0.05 mm. beneath the ependyma. The spike potential (SP) was altered as the recording electrode was inserted into the hippocampus (see columns A and A′). At the ependymal surface, the response was a small spike potential 1–2 msec. duration (fast spike). At the level 0.3–0.4 mm. beneath the surface, a negative spike potential of 2–3 msec. duration (slow spike) appeared. The potentials obtained at a depth of 0.4 mm. consisted of two negative spikes. With further insertion, the slow spike increased in amplitude, and the fast one disappeared probably because it fused with or was masked by the slow spike. The maximum amplitude of the slow spike was attained at a depth 0.4–0.6 mm. (0.6 mm. in this case). The difference between the fast spike and the slow spike is more clear-cut

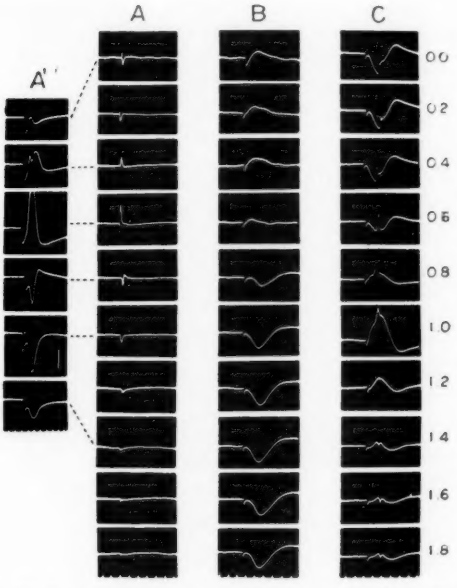

Fig. 3. Various types of response according to depths: Fast and slow spikes (A and A'), slow wave (B) and wave and spike-like potential (C). Depths of recording electrode is indicated in mm. on right side of each record. Stimulating electrode was fixed at a depth of 0.05 mm. for A and A', of 0.3 mm. for B and of 0.8 mm. for C. Time signal: 200/sec. for A, B and C; 1000/sec. for A'. Vertical bar: 1 mV.

in the example shown in Fig. 4. On advancing the electrode further, a phase reversal took place, the negative spike potential changing into a positive deflection, which continuously decreased in amplitude with further insertion, and finally disappeared at a depth 1.6 mm. beneath the ependymal surface.

In column B of Fig. 3 is indicated the laminar distribution of the slow wave (SW). In this case stimulation was delivered at a depth 0.3 mm. beneath the surface. The SW recorded at the surface was a negative one. The wave reversed in sign at a depth 0.7 mm. beneath the surface, and changed into a positive wave which could be recorded even at a level deeper than 2.0 mm. beneath the surface.

When stimulation was carried out at a level 0.8 mm. beneath the surface, slow waves with a spike-like potential were observed at various depths as illustrated in column C. The slow positive wave recorded at the surface was followed by a negative one and reversed its sign at a depth 0.7 mm. beneath the surface. Below that depth, the negative component became

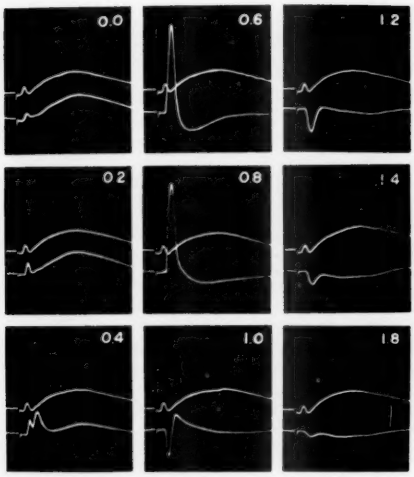

Fig. 4. Fast and slow spikes at different depths. Numerals denote depth in mm. Time signal: 1000/sec. Vertical bar: 1 mV.

dominant. The spike-like potential which was superimposed on the slow wave increased in amplitude on advancing the microelectrode into the deeper layer, and attained a maximum at a depth 0.8 mm. beneath the surface. With further insertion, the spike-like potential decreased rapidly in amplitude, and finally a sign reversal occurred at a depth of 1.3 mm. The layer at which the spike-like potential became maximal did not coincide with the one at which the slow spike was maximal, but the distance between both layers was not so great.

Investigation on the spike potential (*SP*)

From the experiment stated above, it was found that there were two types of spike, that is, fast and slow ones. In order to investigate the difference between the two types of spike, recovery cycles of them were investigated with paired stimuli at various intervals. An example of the records is shown in the left column of Fig. 5, and the recovery curves are shown on the right. As can be seen in the curve connecting dots, the fast spike conditioned by a preceding stimulus recovered its amplitude rapidly and complete recovery was attained in a few msec. The recovery of the slow spike was a little slower as indicated by the curve marked by circles.

Next, the dependence of the slow spike on the stimulus intensity was investigated. As shown in A of Fig. 6, the amplitude of the slow spike increased as the stimulus voltage increased, but the peak latency of the potential was constant regardless of the stimulus intensity.

Fig. 5

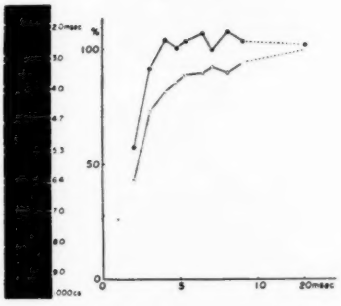

Fig. 6

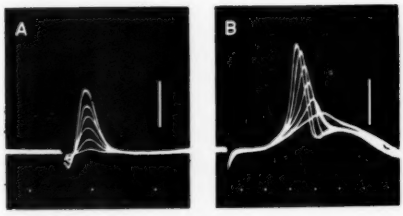

Fig. 5. Recovery cycle of slow spike (circles) and fast spike (dots). Ordinate: Amplitude in percent of that obtained without conditioning stimuli. Abscissa: Interval in msec.

Fig. 6. Dependence of slow spike (A) and spike-like potential (B) on intensities of stimulation (40, 35, 30, 25, 20 and 15 V.). Time signal: 200/sec. Vertical bar: 1 mV.

Further investigation was performed on the effect of drugs upon the spikes. In this and similar experiments, a very tiny pellet of cotton soaked in the agent to be tested was placed between the stimulating and recording electrodes. Recording of responses was made at the surface and the depth where the slow spike was maximum. In these experiments, bipolar stimulation was carried out at the surface through silver electrodes insulated except the tips with a separation of 0.5 mm. Then, both SP and SW were recorded simultaneously.

The effect of 1% procaine dissolved in Ringer's solution upon the spikes was illustrated in Fig. 7. The slow spike was gradually reduced in amplitude in association with similar reduction of the fast spike, and became undetectable a few minutes after the application of the drug (Fig. 7 E). Washing the ependymal surface with Ringer's solution resulted in recovery of the spikes (Fig. 7 F).

The application of 0.3% *d*-tubocurarine solution did not give any effect to the spikes (Fig. 11).

Investigation of the slow wave (SW)

The duration and form of the SW are very similar to those of the so-called dendritic potential observed at the cerebral cortex.[3] Then, the question arose as to whether or not they were similar concerning the other properties.

Records were taken with the recording surface electrode separating 1 mm., 2 mm., 3 mm. and 4 mm. from the site of insertion of the stimulating

Fig. 7

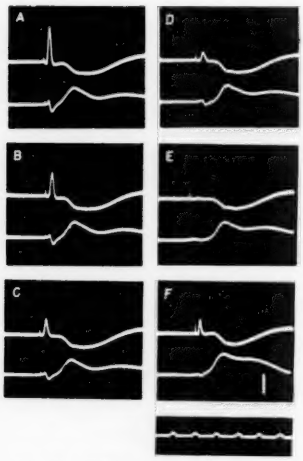

Fig. 8

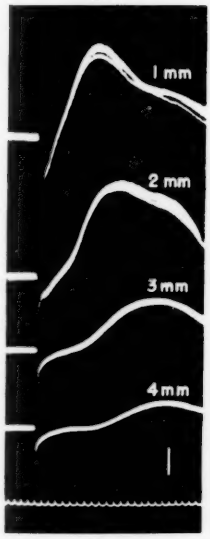

Fig. 7. Effect of procaine on spike and slow wave recorded at ependymal surface (upper line of each record) and at depth (lower line). A: Control response before application of procaine. B, C, D and E: 40, 80, 120 and 160 sec. after procaine application respectively. F: 5 min. after washing surface with Ringer. Time signal: 100/sec.

Fig. 8. Slow waves recorded at certain point of surface evoked by stimulation delivered at different distances indicated on each record. Time signal: 1000/sec.

electrode which was fixed at a certain depth so as to evoke a SW alone. An example is shown in Fig. 8. As seen in this figure, the latency of the SW became longer with increasing separation of the recording electrode from the stimulating one. Calculated from these records, the SW seems to travel at a velocity of 0.5 m./sec., which is of the same order as the conduction velocity of the dendritic potential at the neocortex.[3)4)]

The recovery cycle of the SW was investigated with two equal stimuli at various intervals. SWs recorded at the ependymal surface and a depth 0.5 mm. beneath the surface recovered to the control value within about 20 msec., and showed further increase in amplitude until the maximum enhancement was attained in about 50 msec. The enhancement lasted as long as 500 msec. (Fig. 9).

A topical application of 2% γ-aminobutyric acid (GAB) caused the reversal in phase of the SW recorded at the surface, but the SW recorded at a depth 0.5 mm. beneath the surface was not affected by the drug

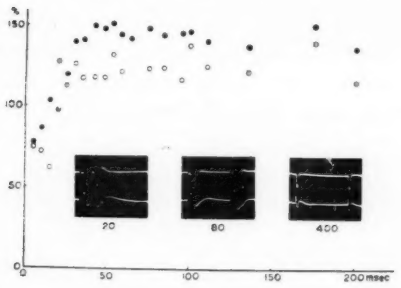

Fig. 9. Recovery cycle of slow wave recorded at surface (dots) and at depth (circles).

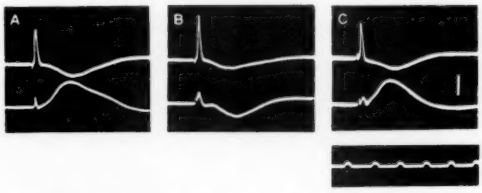

Fig. 10. Effect of GAB on slow wave and spike recorded at surface (lower line) and depth (upper line). A: Before application. B: 1 min. after. C: 30 min. after washing ependymal surface. Time signal: 100/sec.

(see Fig. 10). The reversal in phase occurred within 1 min. after application of GAB. Washing the ependymal surface with Ringer caused recovery of the original wave form (Fig. 10 C). This effect seems to be in accord with the effect of GAB upon the dendritic potential at the neocortex.[5]

Next, the effect of 0.3% *d*-tubocurarine upon the SW recorded at the surface will be mentioned. As illustrated in lower records of Fig. 11, some late components appeared in addition to the usual SW. However the SW recorded at the depth (see upper tracings) became more and more obscure and instead a few spike-like potentials appeared corresponding in time with the surface negativity of the SW. The augmentation and prolongation of the SW observed at the surface may be compared with a similar phenomenon observed about the cortical dendritic potential.[6]

Investigation of the spike-like potential superimposed upon the slow wave

Experiments were done on the spike-like potential of a few msec. duration recorded at the depth where the potential had the maximal amplitude.

The amplitude of the potential increased and the peak latency of it became shorter as the stimulus intensity was raised (see Fig. 6 B).

The recovery cycle of the potential was investigated as usual, and it was found that the potential showed a prolonged phase of supernormality lasting about one sec. An example is shown in Fig. 12. The amplitude

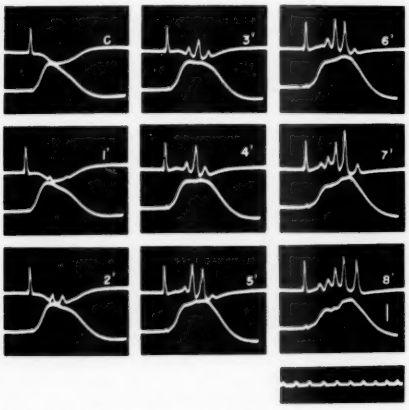

Fig. 11. Effect of *d*-tubocurarine on slow wave and spike. C: Control before application. Time after application is indicated in each record.

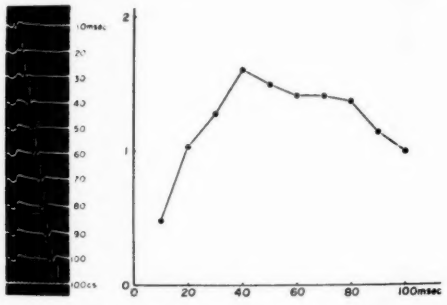

Fig. 12. Recovery cycle of spike-like potential. Ordinate: Amplitude of test response in arbitrary unit. Abscissa: Interval in msec.

of the potential conditioned with the preceding stimulus was enormously high in the supernormal phase as compared with that of unconditioned potential; for example, the amplitude at 40 msec. was over ten times of the control value. The supernormal phase began at several msec. and lasted as long as 1 sec.

In view of the fact that the duration of spike-like potential is comparable

with that of the slow spike potential mentioned above, it is possible that both potentials share an excitable tissue common to them. Therefore some experiments were made on interaction of both potentials. The result is shown in Fig. 13. The latency of the spike-like potential was

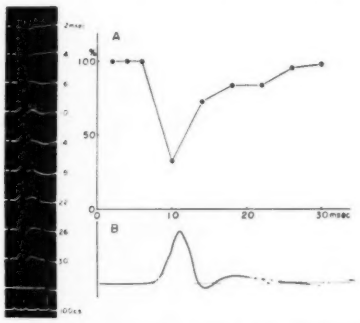

Fig. 13. Interaction between slow spike and spike-like potential. A: Amplitude of test slow spike in percent of control. Abscissa: Time in msec. after conditioning stimulus which evoked spike-like potential. B: Time-course of spike-like potential. Records on left side were used for constructing graph A.

about 5 msec. (see Fig. 13 B), while that of the slow spike was almost zero. Therefore the slow spike preceded the spike-like potential, even when the stimulus for the slow spike was delivered within a period of 5 msec. after the stimulus for the spike-like potential. This relation can be seen in the upper three records in Fig. 13 where the delay is given in msec. by each record. It is without saying that the preceding slow spike was not affected by the conditioning stimulus which evoked the spike-like potential. For greater intervals between the conditioning and test stimuli, the slow spike decreased in amplitude rapidly and reached a minimum at 10 msec. With further lengthening of the interval, the slow spike recovered gradually, and attained the initial level in about 30 msec. (see the curve A in Fig. 13). The spike-like potential was suppressed by the slow spike when closely preceded by the latter (see the left column of Fig. 13). Thus, interaction was found to be mutual.

Discussion

On fast and slow spike potentials

As has been shown above, the fast spike potential was 1 msec. in duration and showed complete recovery within a few msec. after the conditioning stimulus. This finding would lead to a notion that the fast spike represents the activity of nerve fibers. In fact, it is known that in the super-

ficial layer of the hippocampus, *i.e.* the alveus, there are horizontally running dense nerve fibers. Therefore, it is natural to think that the fast spike originated in these fibers in the alveus. Its superficial localization and short latency support this interpretation, too.

The slow spike had its maximal amplitude at the depth corresponding closely to the cell body layer of the hippocampal pyramid (see the right side of Fig. 1). It took longer time to recover for the slow spike conditioned by a preceding stimulus than for the fast spike which reflects the activity of the nerve fibers. During asphyxia it was very difficult to record at a certain definite depth because of the increase in the volume of the brain, and satisfactory estimation of the depth was very difficult, but the following finding seems to be relevant for interpretation of the slow spike. This kind of spike could not be observed at any depth about three minutes after cessation of artificial respiration. This suggests that the slow spike was sensitive to asphyxia. The period of three minutes is of the same order as the asphyxial blocking time of the spinal motoneuron.[7] The facts mentioned above imply that the slow spike represents the activity of the somata of the pyramids.

The question arose as to the relationship between the somata and the axons. In the experiment on the effect of procaine, reduction in amplitude of the fast spike occurred, but the slow wave was not affected by the drug probably because the deeper layer responsible for the slow wave did not suffer from any direct action of the drug. It is, however, to be noted that the slow spike which originated in a far deeper layer was abolished together with the fast spike. The reason for this would be that excitation of the somata represented by the slow spike occurs only indirectly following excitation of the nerve fibers in the alveus.

The next question is whether or not the axons in the alveus and the somata are connected synaptically. The constancy of peak latency of the slow spike during the topical application of *d*-tubocurarine and its independence of stimulus strength suggest that the connection is fairly simple. The same is suggested by the short peak latency of the slow spike and its rapid time-course in the experiment on the recovery cycle. Referring to the Lorente de Nó's divisions in the hippocampus, it is likely that our experiments have been done in regions CA Ib and CA Ic.[8] The hippocampal pyramids in these regions are said to receive no afferent fibers from the alveus. Therefore, the nerve fibers stimulated in our experiments must have been efferent ones. In other wards, the fast spike represents the activity of the axons of the hippocampal pyramids at the alveus and the slow spike represents mainly activity of the somata and partially dendrites fired by antidromic impulses initiated in the axons.

On the slow wave

The slow wave (SW) has properties similar to those of the so-called dendritic potential observed in the neocortex. Anatomically the level of the hippocampus in which the SW is evoked corresponds to the bush of the basal dendrites of the pyramids. Therefore, it is certain that the SW has very close relationship to the basal dendrites.

The recovery cycle of the SW showed a supernormal phase lasting as long as half a sec. Of the recovery cycle of the cerebral dendritic potential, discrepancies were found among the results obtained by different authors: Chang[9] observed a postexcitatory depression, while Clare and Bishop[10] described a phase of depression after facilitation period lasting about 20 msec. In the direct cortical response of the rabbit's cortex,[11] the negative phase of the positive-negative sequence (PN) showed a prolonged supernormal period about 500 msec., while the pure negative deflection (N) showed a prolonged phase of depression. These discrepancies may be due to the difference in either the stimulating technique or the stimulus strength. As we obtained different types of recovery cycle from different cortical regions with one and the same stimulating conditions in our previous experiments,[11] differences in the structure must be another important cause for the different forms of recovery cycle. The long lasting supernormality of the SW may imply that the SW is evoked through a multi-neuronal pathway or pathways.

Cragg and Hamlyn[2] reported that the spike-like potential could be evoked by stimulation of the level of the basal dendrites of the hippocampal pyramids. But we could so far evoke no spike-like potential by stimulation of this level.

On the spike-like potential

The fact that the spike-like potential interacted with the slow spike, resulting in remarkable occlusion of the former, indicates that the potential represents mainly the activity of the somata of the hippocampal pyramids. Long latency more than a few msec. and variability of the peak latency of it suggest that the potential is evoked by the impulses carried over a slow conducting and/or synaptic pathway.

The potential similar to the spike-like potential has been obtained by stimulation of the fornix.[12][13] It is known that an afferent pathway coming through the fornix makes a synapse with the granule cells in the dentate gyrus, and arrives at the hippocampal pyramids. It terminates at the proximal portion of the apical dendrite of the pyramid conducting via the dentate-ammonic pathway. This relation accounts for the fact that similar results are obtained both by stimulation of the fornix and by direct stimulation at the depth corresponding to the apical dendrite level of

the hippocampal pyramid.

Summary

Responses to direct electrical stimulation were recorded at various depths in the hippocampus of the rabbit, and three types of response were distinguished according to the layers stimulated.

1. The first type of response was a spike potential evoked by stimulation at a level 0–0.2 mm. beneath the ependyma. This potential may be divided into two subtypes. One was a small fast spike of about 1 msec. duration recorded at the superficial layer of the hippocampus and the other was a slow negative spike of about 2–3 msec. duration recorded most clearly at a depth 0.4–0.6 mm. beneath the ependyma.

2. The second type was a slow wave evoked by stimulation at a level 0.2–0.4 mm. beneath the ependyma. The wave was negative deflection at the superficial layer of the hippocampus, and phase reversal occurred at a depth of about 0.7 mm.

3. The third type was a slow wave with a spike-like potential evoked by stimulation at a level 0.6–1.0 mm. beneath the ependyma. The slow wave showed a positive negative sequence at the ependymal surface and negative deflection beyond a level 0.7 mm. beneath the ependyma. The negative spike-like potential superimposed upon the wave had a maximal amplitude at about 0.7 mm. depth.

4. The electrophysiological and pharmacological properties of the three types of response were investigated, their relation to the structure of the hippocampus was discussed, and the following conclusion was reached: The fast spike originates in nerve fibers in the alveus, while the slow spike and spike-like potential represent mainly activity of somata of pyramids. The close similarity of the slow wave to the so-called dendritic potential of the neocortex was pointed out.

The author wishes to thank Prof. K. Motokawa for his invaluable discussion and suggestion throughout the course of the experiment and the preparation of the manuscript.

References

1) Suzuki, H. & Taira, N., Jap. J. Physiol. 1958, **8**, 365.
2) Cragg, B. G. & Hamlyn, L. H., J. Physiol., 1955, **129**, 608.
3) Chang, H.-T., J. Neurophysiol., 1951, **14**, 1.
4) Chang, H.-T., Cold Spr. Harb. Symp. quant. Biol., 1952, **17**, 189.
5) Iwama, K. & Jasper, H. H., J. Physiol., 1957, **138**, 365.
6) Chang, H.-T., J. Neurophysiol., 1953, **16**, 221.
7) Lloyd, D. P. C., J. Gen. Physiol., 1953, **36**, 673.
8) Lorente de Nó, R., J. Psychol. Neurol. Lpz., 1934, **46**, 113.
9) Chang, H.-T., J. Neurophysiol., 1951, **14**, 95.

10) Clare, M. H. & Bishop, G. H., EEG clin. Neurophysiol., 1955, **7**, 85.
11) Suzuki, H. & Taira, N., Tohoku J. Exp. Med., 1959, **70**, 1.
12) Campbell, B. & Sutin, J., Amer. J. Physiol., 1959, **196**, 330.
13) von Euler, C., Green, J. D. & Ricci, G., Acta physiol. scand., 1958, **42**, 87.
14) Adey, W. R., Sunderland, S. & Dunlop, C. W., EEG clin. Neurophysiol., 1957, **9**, 309.

The Tohoku Journal of Experimental Medicine, Vol. 71, No. 4, 1960

A Histometrical Study of the Anterior Pituitary Glands of Male Mice from Pre-mature Stage to Puberty

By

Tom Mori, Noriko Fujimori, Akinobu Haga, Shigetaka Murakami
(森　　富)　(藤盛則子)　(芳賀昭信)　(村上成隆)
Seichoku Sugawara and **Yoshikatsu Honda**
(菅原正直)　(本多良勝)

From the Department of Anatomy, Tohoku University School of Medicine, Sendai

(Received for publication, November 16, 1959)

Observations on the histological and cytological changes during the post-natal growth of the anterior pituitary glands of man and of various animals have been made in the past by numbers of investigators. Though only general inspection of the sections without exact enumeration of the cells was apparently made in most of the previous works, it has been generally accepted that with advancing age, the acidophilic and basophilic cells increase in number while the proportion of the chromophobic cells tends to decline. Such a general tendency in the quantitative alterations of the pituitary cells was also observed in the developing mouse pituitary by Yamada[1], Baer[2] and Yamada, Sano and Ito[3] with the exception of somewhat different data of Stein, Caldwell and Peters[4] who described that the percentage of the acidophils was greatest in newborn animals.

According to the recent knowledge on the functional morphology of the pictuitary cells based on the histochemical researches, the post-natal differentiation of the cells, especially the basophils which are regarded to be responsible for the production of certain hormones containing large amount of glycoprotein, is undoubtedly in a close relationship to the sexual development of animals. As for the morphology of the pituitary cells of mouse, however, rather cursory accounts had been made until detailed observations were published by Yamada and his school, especially, Manabe[5], Kato[6], Sano[7][8], and Yamada, Sano and Ito[3]. This paper dealing with a quantitative evaluation of the development of the anterior pituitary cells, especially the basophils, of young male mice has been undertaken as a preliminary survey of the biological standards of the laboratory mouse.

Material and Method

A total of 97 male mice of dd-strain aged from 21 to 63 days were

used. At the 21st day after birth, male youngs were weaned and separated from the female litter mates, and fed a mixed diet containing whole wheat, corn, fish meat and green vegetables until they were sacrificed at every eighth day from the 21st to the 63rd day of age. The pituitary was removed from the cranial bone and the dura mater, and fixed in a mixture of 9 parts of saturated solution of corrosive sublimate and 1 part of formalin for 8–10 hours. The specimen was then imbedded in paraffin and cut serially in the sagittal plane at 3–4 μ.

Most of the sections were stained by McManus'[9] periodic acid—Schiff (PAS) method which was followed by the counterstaining with orange G and hematoxylin. A part of the sections was stained with Gomori's[10] aldehyde-fuchsin (AF) according to the slight modification of Mori, Kato and Fujimori[11].

For counting the pituitary cells, 5 sections along the following sagittal planes were used: 1) the mid-sagittal plane of the gland (plane III illustrated in Fig. 1), 2) the planes through the lateral boundary of the

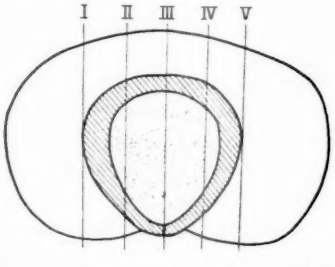

Fig. 1. A schematical illustraiton of dorsal view of the pituitary gland of mouse. The lines I–V represent the sagittal planes selected for the cell-counting and the planimetry of the anterior lobe. The surface of the posterior lobe is stippled, and that of the intermediate lobe is hatched.

intermediate lobe (planes I and V in Fig. 1), and 3) the planes at the middle of the former two (planes II and IV in Fig. 1). As shown in Fig. 2, in the cut surface at the plane III, the anterior lobe forms a thin layer at the bottom of the gland corresponding to the thin median isthmus of the lobe, while the planes I and V cut through the most thick part of the lobe. In the sections at the planes II and IV, the cut surfaces of three lobes of the gland are shown in almost equal breadth. In each of these standard sections, the cell-counting was carried out covering the whole cut area of the anterior lobe under oil immersion. In order to prevent double counting or missing cells, the eye-piece was fitted with Zeiss' "Okularnetzmikrometer," and only cells containing the nucleus were considered in counting.

The same sections used for the cell-counting were projected on a

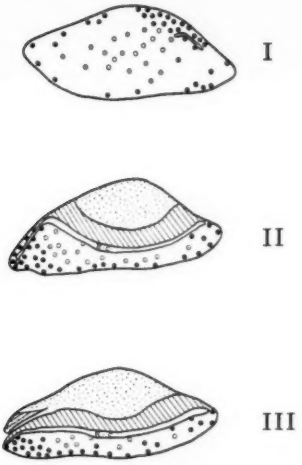

Fig. 2. Typical sections of a 7-week-old male mouse cut at the sagittal planes I, II and III shown in the preceding figure. The cut surface of the infundibular stalk is marked by hatching and stippling. The empty spaces marked R. C. show the residual cleft. In the anterior lobe, distribution of the PAS-positive cells is diagrammatically shown by solid, semi-solid and open symbols representing the intensely PAS-positive cells, the moderately PAS-positive cells and the weakly PAS-positive cells, respectively.

paper in a same magnification (×50), and the area of the cut surface of the anterior lobe was measured with a planimeter.

Observations

1. General morphology of the pituitary basophils in mice

In older mice used in our observation, at least 2 kinds of the PAS-positive basophilic cells were usually found. The cells of the first type were ovoid or sometimes more or less angular in shape and had a round nucleus which contained small number of coarse chromatin particles. The cytoplasm was more or less densely packed with irregularly shaped coarse granules which stained very intensely with PAS. Sometimes, these granules were more densely aggregated in the peripheral area of the cell body. Corresponding to the so-called negative image of the Golgi area, the juxtanuclear region often looked pale lacking the darkly colored granules. The cells of this type were mainly found in the peripheral parts of the anterior lobe (Fig. 2), where they were mostly found in aggregated groups, and especially in the frontal and inferior region of the lobe, they often formed clusters of cells around wide or narrow sinusoidal branches of the pituitary portal vessel. The cells of the second type were

oval and considerably large, and were usually found scattered at random in the central part of the lobe. They were characterized by fine and moderately PAS-positive granules filling up their cytoplasm. Among them, a small number of the cells having faintly stained fine cytoplasmic granules were found intermingled (Fig. 2). But the latter cells were not always clearly distinguishable from those of the second type, and it is still obscure whether they constitute a third type of the pituitary basophils in mice.

In our observation, such a differentiation of the basophils became apparent after 35 days of age. In 21-day-old mice, considerably small cells containing moderately PAS-positive fine granules were found rather evenly throughout the lobe, except in the mid-anterior part of the inferior region where small number of cells containing dark-red coarse granules were occasionally found attached to the portal vessel. In 28-day-old mice, the PAS-reactivity of the granules and the outline of the cell bodies became more definite. In some specimens of this stage, a number of intensely PAS-positive cells were forming cellular clusters in the mid-anterior region of the lobe. These cells contained in their cytoplasm deep-red coarse granules and often deeply colored droplets, too. Some of them already showed a round perinuclear pale area corresponding to the Golgi zone. In the central part of the same pituitaries, a small number of weakly PAS-reactive (pink to pale-red) cells were found.

As for aldehyde-fuchsin staining, only a fairly compatible parallelism was obtained between the affinity to AF and intensity of PAS-reaction, the intensely PAS-positive cells in the peripheral district of the lobe being also darkly colored by AF, and weakly PAS-positive cells in the central district showing a little affinity to AF.

2. Quantitative development of anterior pituitary cells

For the enumeration of the basophils, sections at the planes I–V stained with PAS-orange G-hematoxylin were used, and the comparison was made among total numbers of the basophils counted in the whole areas of these 5 cut surfaces of respective specimens. The minimum, maximum and mean values and the estimated fiducial limit of the mean of such a total number of the basophils at the end of each week is presented in Table I. As obviously represented in the table, the number of the basophils steadily increased until the 42nd day, especially rapidly before the 35th day. A gradual increase in number still continued in the more advanced stages but such a slight rise in each week-interval was not significant. Such a retardation of the increment is also clearly represented by a marked reduction of correlation coefficient (Table III). As shown in Figs. 3 and 4 and Table III, this increase of the basophils was more closely correlated with the body weight than the weekly age of the animals.

The differential cell-count was done by enumerating the three princi-

TABLE I

The Number of the Pituitary Basophils at Different Daily Ages of Male dd-Mice

Age in days	Number of mice	Average body weight	Min.	Max.	Mean ± Confid.* limit (P=5%)	Coeff. of var.	% differ.	P** value
		gm.						
21	9	6.1	310	399	347.4±28.9	10.1		
							57.94	0.001
28	10	8.6	477	639	548.7±45.4	11.0		
							76.33	0.001
35	19	17.1	830	1090	967.5±43.9	9.2		
							8.63	0.05
42	13	19.6	867	1201	1051.0±68.8	10.4		
							4.04	0.3
49	18	21.3	959	1326	1093.5±50.4	9.0		
							6.62	0.2
56	12	21.9	927	1441	1165.9±86.9	11.0		
							5.29	0.4
63	16	23.3	1001	1546	1227.6±94.3	14.0		

* Confidence limit of mean is given by $\frac{t_0 - u}{\sqrt{N-1}}$, where t_0 is the value of t obtained from the table of t-distribution ($\alpha = 0.05$; $n = N-1$) and $u = \sqrt{\frac{\sum (x-\bar{x})^2}{N-1}}$

** P value for the difference between the means. Values less than 0.05 are considered statistically significant.

pal types, the basophils, acidophils and chromophobes, of the pituitary cells in 3 sections at the sagittal planes I–III or III–V. In classifying, the cells without any recognizable cytoplasmic granules were classified as the chromophobes. So, the chromophobes in our classification possibly contain, in part, some undifferentiated cells or degranulated chromophils. The weekly changes in proportions of the three types of cells were summarized in Fig. 5, where each point represents the weighted mean of the percentage of the respective cell-type. In this figure, the different trends shown by the acidophils and the chromophobes, the steady increase in the percentage of the former and the continuous decline in that of the latter, are clearly demonstrated. On the 21st day, the chromophobes constitute nearly 70 per cent of the pituitary cells, while the acidophils represent about one-fourth of all the cells. But at the end of the 6th week, the proportions of these two types of cells are on almost the same level; 48 per cent for the acidophils and 44 per cent for the chromophobes. From the 6th week up to the 8th week there is no significant change in the percentages of both the cells, but after the latter period, a rise in the

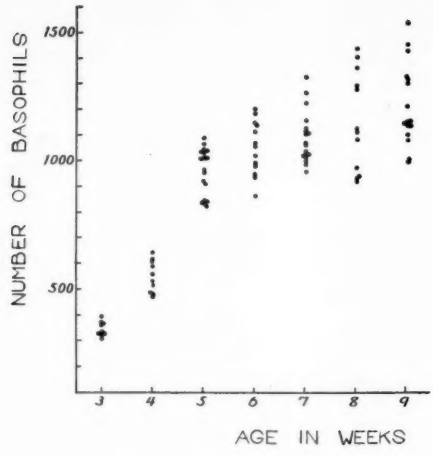

Fig. 3. A scattergram showing weekly change in the number of PAS-positive pituitary basophils.

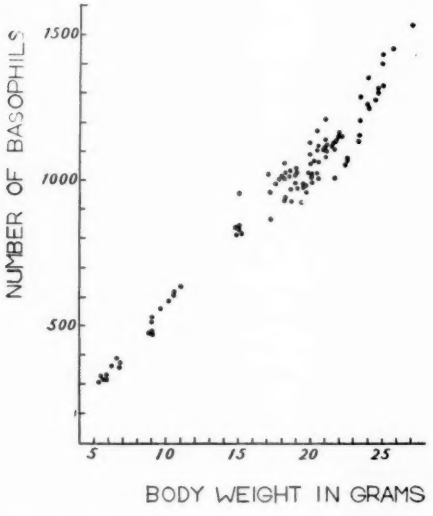

Fig. 4. A scattergram illustrating a linear correlation between the number of PAS-positive basophils and the body weights of 3- to 9-week-old male mice.

percentage of the chromophobes and a fall in that of the acidophils take place, both changes being statistically significant. Such difference in

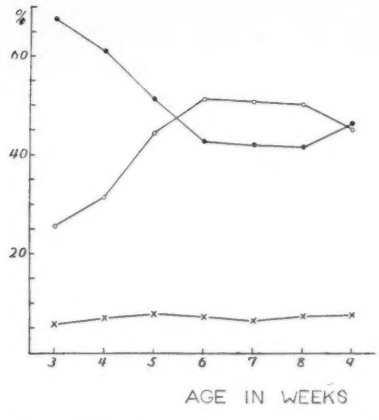

Fig. 5. Weekly changes of the relative numbers of the acidophils (open circle), the chromophobes (solid circle) and the basophils (cross).

trends before and after the 6th week are also clearly represented by the highly significant difference of correlation coefficients (Table III). Acompanying the increase in the percentage, the absolute number of the acidophils showed remarkable increment until the 7th week, but after that, no numerical increase was perceived. As for the chromophobes, notwithstanding the marked decrease in their proportion, their absolute number showed a slow but continuous rise throughout the period of our observation.

The percentage of the basophils on the other hand was the lowest among those of the three types of cells and showed no constant and conspicuous change with growth (Fig. 5), no statistically significant correlation between the percentage and the weekly age being established (Table III). But there was a slight increase from 3 to 5 weeks and a temporal decline between 5 and 6 weeks, both the changes were significant only when the level of significance was settled at 95 per cent or less. The rise in the relative number of the cells in the former stage may be due to the rapid increase in the absolute number of the cells at the same period. For the decrease in the latter week-interval, the marked retardation of multiplication of the cells and the still advancing increase of the acidophils and the chromophobes may possibly be responsible.

3. Growth in size of the anterior lobe

The size of each anterior pituitary was represented by the summed total area of the lobe in the 5 cut surfaces corresponding to the selected sections I–V. In Table II, the weekly changes of the area of the lobe are shown; the general trend of the chronological alteration is in good agree-

TABLE II

The Weekly Change of the Cut Surface Area of the Anterior Pituitary (mm^2)

Age in days	Number of mice	Min.	Max.	Mean ± Confid. limit (P=5%)	Coeff. of var.	% differ.	P value
21	9	0.31	0.52	0.42±0.06	16.6		
						45.2	0.001
28	10	0.49	0.89	0.62±0.09	18.7		
						101.6	0.001
35	19	0.84	1.73	1.25±0.12	18.6		
						17.6	0.02
42	13	1.09	1.91	1.47±0.14	15.6		
						5.4	0.3
49	18	1.26	1.87	1.55±0.09	11.5		
						0	—
56	12	1.21	1.99	1.55±0.05	4.7		
						1.9	0.9
63	16	0.96	2.34	1.58±0.22	25.6		

TABLE III

A Summarized Table of Correlation Coefficients

	Number of Basophils	% Acidophils	% Basophils	% Chromophobes	Cut surface area of ant. lobe
Weekly age					
3rd-9th weeks	0.79	0.70	0.19*	−0.69	0.71
3rd-6th weeks	0.91	0.86	—	−0.81	0.88
7th-9th weeks	0.35	−0.43	—	0.38	0.12*
Body weight	0.95	0.78	0.31	−0.83	0.87

* The Correlation Coefficient (ρ) was estimated as statistically 0 at the 5% level of P.

ment with that of the number of basophils, and as in the case of the basophils, the increase of the lobe is also more closely related to the increase of the body weight than to the weekly age (Table III). The population of the basophils per unit area of the anterior lobe was also calculated, but no noticeable change was obtained throughout the period of observation.

DISCUSSION

With the aid of two histochemical staining procedures, the periodic acid-Schiff technique of McManus[9] and the aldehyde-fuchsin stain of Gomori[10], our knowledge on the morphology and the function of the pituitary basophilic cells has been greatly advanced recently. In 1949,

Catchpole[12] and Pearse[13] introduced the PAS technique to the study of the hypophysis, and the positive reactivity to this staining of the basophilic granules was confirmed. The latter author,[14] furthermore, divided the basophils into " maximal " and " intermediate " mucoid cells according to the stainability of the granules, such a difference in PAS-reactivity, he thought, being dependent on the amount of mucoprotein contained in these granules. As for the aldehyde-fuchsin staining, Gomori[10] pointed out that his new stain selectively colored the cytoplasmic granules of a certain type of the pituitary basophils. This observation was confirmed later by Halmi[15-18] who distintuished two types of basophils, AF-positive beta cells and AF-negative delta cells, in rat and mouse hypophyses, and suggested that these two have thyrotrophic and gonadotrophic activity, respectively. Studies on such a functional significance of the beta and delta cells were comprehensively extended by Purves and Griesbach[19-21]. Using the rat hypophysis, they introduced new functional appelations, the " thyrotrophic cells " for the beta cells and the " gonadotrophic cells " for the delta cells, and they further subdivided the latter cell type into the " peripheral " and the " central gonadotrophs "[22][23].

Detailed description on the morphology of the mouse pituitary cells has been presented recently by Yamada and his collaborators[3][5-8]. According to them, there are two types of basophils in mouse, the one corresponding to the delta cells and the other to the beta cells. The cells of the first type are oval or polygonal and their cytoplasm contain fine granules which are intensely PAS-positive but AF-negative. They mainly distribute in the frontal region including the sex-zone and the pars tuberalis, the inferior region, and the superior region facing the residual cleft. Those of the second type are large and polygonal or irregular in shape, and are mainly found in the central part of the anterior lobe. The cytoplasmic granules are coarse and are darkly colored with AF, and also stain pink by PAS-method.

In our observation, too, PAS-positive basophils of dd-mouse consisted of two types, which are apparently corresponding to the first and the second types of Yamada's classification, but strongly PAS-positive granules of the delta cells were looked more coarse than weakly or moderately PAS-positive beta granules in our preparations. Contrary to the findings of most investigators, the affinity to AF and the reactivity to PAS of the basophils in our preparations were found in fairly good parallelism. Although further critical examinations should be done for such a parallel stainability, an impression was gained in this work that the morphological discrimination of two sub-types of the pituitary basophils in mouse was less easy than in the case of rat.

The differentiation of these two types of basophils became apparent

after 35 days in our mouse but a sign of definite localization of the intensely PAS-positive cells was already evident in the specimens aged 21 days. This suggests that the delta cells precede the beta cells in development, as was pointed out by Siperstein, Nichols, Griesbach and Chaikoff[24] in their detailed observation of developing rat anterior hypophysis.

Our numerical observation has confirmed the weekly increase in number of the basophils, especially in younger animals before 42 days. This increase is more closely correlated to the body weight than the weekly age of the animals, unlike the finding of Phillips and Piip[25] who could not detect consistent relationship between the body weight and the number of AF-positive cells in rats ranging from birth to 15 days. In the percentage of the basophils, in general, there was no significant correlation between the percentage and the weekly age. This invariability in relative number of the cells is very similar to Rasmussen's[26] observation on man.

This study also tends to substantiate the generally accepted trends in the chronological changes of the proportions of the acidophils and the chromphobes, a steady increase of the former and a continuous decline of the latter. It should be noticed here, however, that such a well-known tendency was proved only until the 6th week. The increase in size of the anterior lobe was also restricted within the younger stage before the end of the 6th week.

From these findings, it is obvious that at the end of the 6th week, very remarkable and abrupt changes in the development in number and/or proportion of respective types of the anterior pituitary cells take place synchroneously. It seems very possible that such a concurrent change in the quantitative development is closely related to the sexual maturity of the animal. This view may be supported by the fact that the vaginal orifice of female mice of the same strain bred in our laboratory under the same environment become established on the 44th day of age on the average.

Summary

1. The development of the anterior pituitary cells of male mice from pre-mature stage to puberty was studied histometrically. For the cell-counting, sections cut along the selected sagittal planes and stained with PAS-orange G-hematoxylin were used.

2. According to the staining property and the localization, the existence of two types of PAS-positive basophils corresponding to the beta and the delta cells was confirmed.

3. The number of the basophils and the percentage of the acidophils showed steady rise until the end of the 6th week, while the percentage of the chromophobes fell abrest during the same period. The percentage of the basophils remained almost constant. The growth in size of the anterior

lobe was also found in the younger stage before the end of the 6th week.

4. From these findings, it was pointed out that a remarkable retardation in the quantitative development of the anterior pituitary takes place at the end of the 6th week in the male mice of dd-strain.

References

1) Yamada, Nagoya Igakkai Zassi (Jap.), 1937, **46**, 983.
2) Baer, Acta Neerl. Morph. Norm. et Path., 1942, **4**, 233 (cited by Biol. Abstr., 1948, **22**, Abstr. No. 23720).
3) Yamada, Sano and Ito, Folia anat. jap., 1957, **30**, 177.
4) Stein, Caldwell and Peters, Anat. Rec., 1942, **84**, 523.
5) Manabe, Nagoya Igaku (Jap.), 1956, **71**, 8.
6) Kato, Nagoya Igaku (Jap.), 1956, **72**, 489.
7) Sano, Folia anat. jap., 1956, **29**, 247.
8) Sano, Folia anat. jap., 1958, **31**, 17.
9) McManus, Nature, 1946, **158**, 202.
10) Gomori, Am. J. Clin. Path., 1950, **20**, 665.
11) Mori, Kato and Fujimori, Tohoku J. Exp. Med., 1958, **68**, 1.
12) Catchpole, J. Endocrinol., 1949, **6**, 218 (cited by Yamada *et al.* [3]).
13) Pearse, J. Path. Bact., 1949, **61**, 195.
14) Pearse, J. Path. Bact., 1950, **64**, 811.
15) Halmi, Endocrinol., 1950, **47**, 289.
16) Halmi, Anat. Rec., 1952, **109**, 300.
17) Halmi, Stain Techn., 1952, **27**, 61.
18) Halmi, Endocrinol., 1952, **50**, 140.
19) Purves and Griesbach, Endocrinol., 1951, **49**, 244.
20) Purves and Griesbach, Endocrinol., 1951, **49**, 427.
21) Purves and Griesbach, Endocrinol., 1951, **49**, 652.
22) Purves and Griesbach, Endocrinol., 1954, **55**, 785.
23) Purves and Griesbach, Endocrinol., 1955, **56**, 374.
24) Siperstein, Nichols, Griesbach and Chaikoff, Anat. Rec., 1954, **118**, 593.
25) Phillips and Piip, Anat. Rec., 1957, **129**, 415.
26) Rasmussen, Am. J. Anat., 1950, **86**, 75.

The Tohoku Journal of Experimental Medicine, Vol. 71, No. 4, 1960

Preparation of Serial Carbowax Sections for Fat-Staining : A Combined Gelatin-Carbowax-Paraffin Technic

By

Tom Mori, Toshi-hiro Ishii and **Akinobu Haga**
(森　　富)　(石井敏弘)　　(芳賀昭信)

From the Department of Anatomy, Tohoku University School of Medicine, Sendai

(Received for publication, November 16, 1959)

The water-soluble polyethylene glycol wax known as carbowax (Carbide and Carbon Chemicals Corporation, New York) is now widely used in routine histological procedures as a dehydrating and imbedding agent. The carbowax-method greatly shorten the process and is especially advantageous when small specimens which are too small to cut with a freezing microtome are to be fat-stained. As is well-known, however, the brittle and hygroscopic character of carbowax not infrequently makes the sectioning and subsequent manipulation of the sections very difficult. In order to overcome such technical difficulties, varieties of improvements or modifications were reported by many investigators. Among them, according to our experience, an imbedding in paraffin of the specimen after carbowax bath introduced by Shinotô, Hoshi and Matsunaga[1] and Goland, Jason and Berry[2], technics introduced by these two groups of authors being slightly different in details, is most satisfactory to get not curled, well-cut sections in the form of continuous ribbon. But, if an observation on a complete serial sections is required, although the labor for serial sectioning is greatly lightened with the aid of this combined carbowax-paraffin technic, it is still cumbersome to mount those sections on the slides serially just as they were ordered in the ribbon. By routine albumen-water method, the carbowax sections do not firmly stick on the slide and they often tend to detach from the latter during the subsequent staining procedure. In our laboratory this difficulty has been obviated by employing a thin solution of gelatin to stretch the sections on the slide and by exposing the slide to formalin vapor thereafter in order to assure the adhesion of the sections as Gömöri[3] did in his silver-impregnation method. Moreover, we found immersion of the specimen in a gelatin solution prior to the carbowax bath was very satisfactory to prevent de-

struction of the sections during the stretching. This combined carbowax-paraffin technic preceded by gelatin infiltration has enabled us to perform fat-staining with sudan dyes on a complete series of carbowax sections. In this paper, the schedule of this technic is presented.

All of the tissues used were fixed and stored in 10% formalin. As for the carbowax, those of 1500 and 4000 of molecular weight were employed. None of the fatty-acid esters of polyethylene glycol recommended by Carsten[4] and Goland *et al.*[2] was used, since Lillie[5] suggested that such esters may cause some loss of lipids from the tissues.

Description of the Technic

1. Gelatin infiltration: The specimens fixed in 10% formalin are thoroughly washed in running water for about 24 hours, and then immersed in 5% solution of gelatin in 1% phenol for about 24 hours at 37°C. We usually change the gelatin solution two times during this step.

2. Hardening gelatin: The specimens are picked out from the warm gelatin solution and transferred into chilled 10% formalin in a glass tube which is gently shaken so as to remove excess gelatin from the surface of the specimens. Then the tube is left overnight at room temperature to harden the gelatined tissue.

3. Carbowax infiltration: After a thorough washing under the tap, the hardened specimens are dipped into molten carbowax. In our laboratory, the specimens are passed through carbowax 1500, an equal mixture of carbowaxes 1500 and 4000, and then pure carbowax 4000, this schedule being generally used in Japan according to the method of Ono and Shida[6]. The infiltration in these successive carbowax baths has been kept up for about 30, 30–60, and 60 minutes, respectively, but some organs such as the testis usually required longer duration.

4. Paraffin imbedding: The specimens soaked with carbowax are just rinsed in two pots of molten hard paraffin, and imbedded immediately in paraffin. The paraffin should be solidified at room temperature or in a refrigerator, but not in water. The solid mass of paraffin is trimmed leaving a considerably thick coat of paraffin around the specimen.

5. Mounting sections on the slide: At first, the slide is warmed on a heated plate adjusted at 55–56°C., and an appropriate amount of very thin gelatin water which is prepared by 8–32 times dilution of the above-mentioned gelatin-phenol solution used in the preliminary infiltration is added on the slide. The ribbon of sections is cut in segments containing 1–2–3 sections according to their size. Then the segments are carefully put on the warm gelatin water. The rim of paraffin encircling each section spreads on the surface of the water forming a large space, in which the tissue section gradually spreads and settles on the surface of the slide. After

the sections have stretched and flattened enough, the excess gelatin is blotted off from the outside of the paraffin without tilting the slide. When the specimen is small, we usually put 6 to 12 sections on one slide. After this procedure, the slides are stored in a well-sealed staining vessel containing a piece of cotton soaked with formalin, and the vessel is allowed to stand in an incubator adjusted at 37°C. for at least one night.

In this procedure, the temperature of the gelatin water should be most carefully controlled. When the temperature is too low, the rim of paraffin does not extend well, so that the peripheral part of the stretched section is covered by paraffin. On the other hand, when the gelatin water is too hot, the section will be coated by a thin film of melted paraffin. If desired, the rim of paraffin may be taken off gently with a pair of fine forceps while it is floating on the gelatin water, though this procedure is rather cumbersome to perform without damaging the sections, and is not necessary unless the sections are covered by paraffin.

Discussion

As described above, the final imbedding in paraffin of carbowaxed specimen seems very effective to avoid technical difficulties depending mainly on brittleness and hygroscopicity of the carbowax. According to Wade[7], however, the so-called carbowax 1500 is a mixture of polyethylene glycol 300 and carbowax 1540, the hygroscopicity of the latter being only 1/20 of that of glycerol, while that of the former compound being as high as glycerol. Better results may therefore be expected by replacing the carbowax 1500 in our technic by carbowax 1540 but the latter compound was not yet available until this paper has been completed.

In order to assure a regular preparation of good sections, a soak of the carbowaxed specimen in hot xylene prior to the final imbedding in paraffin (Goland *et al.*[2]) is most recommendable. Using this schedule we could successfully make good sections of such organs as the adrenal gland accompanying a thick capsule of the adipose tissue, the sectioning of the latter being almost impossible by routine carbowax technic. But, if fat-staining is desired, the xylene bath should be avoided because it results in a severe loss of fat from the specimen. So, according to our experience, the larger amount of the adipose tissue encapsulating some organs should be removed mechanically before the preliminary infiltration in gelatin.

Other difficulties in the carbowax method are destruction of the sections on water and inadequate affixing of them on the slide. Many attempts have been made in the past for preventing destruction of the sections, and special attention of many investigators such as Firminger[8], Suzue and Ichida[9], Wade[7], and Zugibe, Kopaczyk, Cape and Last[10] (" Special Flotation

Solution ") has been centered on to depress the surface tension of water which is regarded most responsible for the destruction of fragile carbowax sections. Solutions containing gelatin were also used by some authors as a flotant (Blank and McCarthy[11], Zugibe *et al's*[10] " General Flotation Solution ") or as an affixative (Rinehart and Abul-Haj[12], Giovacchini[13]). Rinehart and Abul-Haj[12] and Giovacchini[13] used formalin and gelatin in combination, and their methods have something common in this respect with our method.

During the course of our trials, we found that a thin solution of polyvinylpyrrolidone (PVP, General Aniline and Film Corporation, New York) was an excellent flotant to get well-stretched sections without any damage. But this compound was not suitable to stretch the sections on the slide, since its hygroscopicity seemed to interfere with the adhesion of the sections. The very thin (approximately 0.6–0.15%) solution of gelatin used in our technic is highly satisfactory because it does not harm the sections and keeps the sections very firmly on the slide after the treatment with formalin vapor.

Summary

A modification of combined carbowax-paraffin technic including a preliminary infiltration of the specimen in gelatin is presented. This gelatin-carbowax-paraffin technic yields serially arranged and firmly adhered sections which may be used for staining fat with sudan dyes.

References

1) Shinotô, Hoshi and Matsunaga, Nippon Iji Shimpô (Jap.), 1951, No. 1437, 16.
2) Goland, Jason and Berry, Stain Techn., 1954, **29**, 5.
3) Gömöri, Am. J. Path., 1937, **13**, 993.
4) Carsten, Arch. Path., 1947, **44**, 96.
5) Lillie, Histopathologic technic and practical histochemistry, 1954, Blakiston Co. Inc., New York and Toronto.
6) Ono and Shida, Sôgô Igaku (Jap.), 1950, **7**, 130.
7) Wade, Stain Techn., 1952, **27**, 71.
8) Firminger, Stain Techn., 1950, **25**, 121.
9) Suzue and Ichida, Sôgô Igaku (Jap.), 1951, **8**, 858.
10) Zugibe, Kopaczyk, Cape and Last, J. Histochem. Cytochem., 1958, **6**, 133.
11) Blank and McCarthy, J. Lab. Clin. Med., 1950, **36**, 776.
12) Rinehart and Abul-Haj, Arch. Path., 1951, **51**, 666.
13) Giovacchini, Stain Techn., 1958, **33**, 247.

The Tohoku Journal of Experimental Medicine, Vol. 71, No. 4, 1960

Idiopathic Hypercalcemia of Infancy

By

Tooru Nakao, Toshio Nitta and **Takao Gotooda**
(中尾 亨) (新田敏雄) (後藤田隆雄)

From the Department of Pediatrics, Aomori Prefectural Central Hospital; Pediatrician-in-Chief: Dr. T. Nakao

(Received for publication, November 16, 1959)

The disease called " Idiopathic hypercalcemia of infancy " is clinically characterized by growth failure, anorexia, constipation, vomiting and a peculiar face. Laboratory studies disclose hypercalcemia and elevating of blood urea. Butler[1] and Fanconi[2][3] reported the first two cases in 1951. In 1952, the syndrom was clearly delineated by Lightwood and Payne[4] for the first time. Idiopathic hypercalcemia of infancy has thus far been discussed mainly in Great Britain,[1][4][5-17] but recently in United States[18-20] several cases have been reported. In the present paper we shall describe the first report of this disease in Japan.

Report of Cases

Case 1. The boy weighed 3.75 kilograms at birth, April 24, 1956. His first visit to our Clinic was at the age of 3 months because of obstinate vomiting and constipation.. At the age of 4 months, he was admitted to our Hospital as a suspected case of invagination because of frequent vomiting, but he improved with transfusion and was discharged. But thereafter vomiting and constipation persisted in spite of various treatment, and he was often attacked with fever. At the age of 1 year and 9 months, he was readmitted to the Hospital because of measles. Though the course was severe, he recovered from the disease at length. But by this time, he had already had a peculiar face (Fig. 1). He was malnourished, hypotonic and his mental development was delayed. Heart sounds were clear. From the time of readmission to his full age of 2 years biochemical examinations of serum were performed. As is given in Table I, serum calcium level was elevated. Serum nonprotein nitrogen was slightly elevated, but serum phosphorus level, alkaline phosphatase activity and cholesterol level were within normal ranges. Roentgenogram of the bones showed dense lines at the end of the long bones and a slight osteosclerosis. Calcification was not found in the kidneys. He had been fed on cow's milk from his birth, but he had not vitamin D intake specially. At the age of 2 years

Fig. 1. Case 1. Elfin appearance with a big mouth and thickened upper lip.

TABLE I

Biochemical Findings of Serum of Our Own Cases of Idiopathic Hypercalcemia of Infancy

	Calcium (mg/dl)	Phosphorus (mg/dl)	Alkaline phosphatase activity (S. J. R. unit)	Nonprotein nitrogen (mg/dl)	Cholesterol (mg/dl)
Case 1	11.6-14.5	5.5	4.1- 4.8	27.0-40.8	176
Case 2	9.7-13.6	2.5-8.2	2.5-12.7	21.0-49.0	204
Case 3	10.1-14.0	5.0-6.0	4.7-16.8	28.0	—

and 1 month, he could not yet stand still.

Case 2. The girl weighed 3 kilograms at birth September 24, 1947. She suffered from persistent vomiting and constipation from the age of about 5 months. She was admitted to the Hospital on March 3 and remained to stay until April 4, 1958, because of bronchopneumonia. On the examination at this time, slight systolic murmurs were heard on the apex. Strabismus was noticed. From then on systolic murmurs became louder and louder and strabismus was more definite. Constipation and vomiting were very obstinate. She was readmitted to the Hospital on July 3 and stayed in it until September 12, 1958, because of moniliasis of the lungs due to candida albicans. During her admission and even later, she was often attacked with fever. Biochemical examinations of serum were performed repeatedly from January to August, 1958. As is given in Table I, serum calcium level and nonprotein nitrogen were elevated. Blood picture was normal except for a slight anemia. Sulkowitch

test and protein reaction of the urine were positive on July 4, 1958. Roentgenogram showed a slightly increased density of the bones and dense lines at the end of the long bones, but no calcification of the kidneys was found. She had been fed on cow's milk and in addition received 0.5 cc. multivitamins solution containing 500 units of vitamin D_2 per cc. daily. But since her first admission, the multivitamins solution intake had been suspended. On the examination at the age of 2 years, September 20, 1959, heart sounds were clear and serum calcium level was reduced to normal, but she could not walk yet. Strabismus was still noticed.

Case 3. The boy weighed 3.8 kilograms at birth, June 5, 1957. He was admitted to the Hospital on July 12, 1957, because of persistent fever. After admission, he was treated as a suspected case of sepsis. Blood cultures were repeatedly performed, but the results were negative. The unexplained fever persisted. He began to vomit from the age of about 2 months on, and vomiting became more and more frequent. Constipation also was very obstinate. His face presented a peculiar appearance (Fig. 2), which became gradually striking. Serum calcium level was

Fig. 2. Case 2. Elfin appearance with a big mouth and hypertelorism.

14.0 mg./dl. on the 12th day of his admission. Dehydration and thirst were noticed. He was hypotonic. He was discharged with no remarkable improvement on October 10, 1957. During his admission, the increase of his body weight was very slow and he gained only about 1 kilogram during the time of 3 months. Blood picture was normal except for a slight anemia. Red sedimentation rate was not accelerated. Roentgenogram showed an increased density of the bones and dense lines at the end of the long bones. Slight calcification was found in the kidneys. He had been fed on both mother's and cow's milk since his birth, but he had

not vitamin D intake specially. Nonprotein nitrogen level at his discharge was within normal range.

Comment

Our own cases belong to the severe form of idiopathic hypercalcemia of infancy such as has been described by Lightwood and Stapleton.[6] They presented each the classic "elfin appearance" with a big mouth and a thickend upper lip. Anorexia, constipation, vomiting, very slow gain or standstill of weight, growth failure and mental impairment were typical. In Case 2, systolic murmurs were heard over her heart. Serum calcium levels in all the three cases were elevated. This is, as is well known, the most important finding for the diagnosis of this syndrome: "Idiopathic hypercalcemia of infancy." Nonprotein nitrogen level was also elevated except in Case 3, but in this case the estimation was performed at the time of his discharge, the possibility that his nonprotein nitrogen level might, if examined much more early during his stay, have been found to be elevated, is not excluded. Roentgenograms of the bones in all the cases showed dense lines at the end of the long bones. In Case 3, slight calcification was found in the kidneys. As to the etiology of this syndrome, nothing certain is known. Individual hypersensitivity to vitamin D has been suggested as a possible etiology by many authers.[7][8][21-23] Case 1 and Case 3 did not receive any additional vitamin D intake. In Case 2, 250 units of vitamin D were indeed daily given, but this dosis is by no means an excessive one.

Summary

Three cases of idiopathic hypercalcemia of infancy were presented. The present paper is the first report of this syndrome in Japan. In all the cases the typical clinical features such as anorexia, constipation, vomiting, growth failure and mental impairment were noticed. Serum calcium levels were elevated in all the cases. Nonprotein nitrogen level was elevated except in one case.

References

1) Butler, Proc. Roy. Soc. Med., 1951, **44**, 296.
2) Fanconi, Schweiz. med. Wschr., 1951, **81**, 908.
3) Fanconi, Helvet. paediat. acta, 1952, **7**, 314.
4) Lightwood and Payne, Arch. Dis. Childh., 1952, **27**, 302.
5) Creery, Lancet, 1953, **2**, 17.
6) Lightwood and Stapleton, *ibid.*, 1953, **2**, 255.
7) Lowe, Henderson, Park and McGreal, *ibid.*, 1954, **2**, 101.
8) Creery and Neil, *ibid.*, 1954, **2**, 110.
9) Dauson, Craig and Perera, Arch. Dis. Childh., 1954, **29**, 475.

10) Schlesinger, Butler and Black, Brit. M. J., 1956, **1**, 127.
11) Forfar, Balf, Maxwell. and Tompsett, Lancet, 1956, **1**, 981.
12) Morgan, Mitchell, Stowers and Thompson, *ibid.*, 1956, **1**, 925.
13) Stapleton, McDonald and Lightwood, *ibid.*, 1956, **1**, 932.
14) Rhaney and Mitchell, *ibid.*, 1956, **1**, 1028.
15) Russell and Young, Proc. Roy. Soc. Med., 1954, **47**, 1036.
16) Stapleton and Evans, Helvet paediat. acta, 1955, **10**, 149.
17) Schlesinger, Butler and Black, *ibid.*, 1952, **7**, 335.
18) Daeschner and Daeschner, Pediatrics, 1957, **19**, 362.
19) Bongiovanni, Eberlein and Jones, New England J. Med., 1957, **257**, 951.
20) Snyder, Am. J. Dis. Child., 1958, **96**, 376.
21) Fanconi and Spar, Helvet. paediat. acta, 1955, **10**, 156.
22) Korányi, Mschr. Kinderheilk., 1958, **106**, 381.
23) Shiers, Am. J. Roentg. 1957, **78**, 19.

The Tohoku Journal of Experimental Medicine, Vol. 71, No. 4, 1960

Meningoencephalitis due to Influenza Virus B

By

Tooru Nakao
(中尾　亨)

From the Department of Pediatrics, Aomori Prefectural Central Hospital; Pediatrician-in-Chief: Dr. T. Nakao

(Received for publication, November 16, 1959)

Many cases of neurological disorders during epidemics of influenza have been reported since Leichtenstein's[1] description, but cases caused by influenza virus, confirmed serologically or by means of virus isolation, were only a few in number until 1957.[2-4] However, in the recent epidemic of Asian influenza, many cases with disorders of the nervous system were reported with virological confirmation.[5-11] From the end of April to May, 1955, in Aomori City and its neighborhood, an epidemic of influenza was prevalent. Laboratory investigation revealed that this epidemic was caused mainly by influenza virus A, though there were scattered cases of influenza B and of influenza A-prime. In the present paper I shall report the first case of meningoencephalitis due to influenza virus B in Japan which was confirmed serologically.

Report of Case

A boy, aged 6 years and 10 months, complained of high temperature and headache from May 3, 1955. He was treated by a physician for 3 days and fever abated slightly on May 8, but then anorexia and lethargy persisted. On May 11, he had convulsive fits twice before noon and he was admitted to our Hospital. He had never vomited until this time. On examination, no abnormality was found in the lungs and abdomen. Heart sounds were clear, but a slight bradycardia was noticed. Patellar reflex diminished, but nuchal rigidity was noticed. He was unconcious and treated as a case of meingoencephalitis on the basis of clinical features and laboratory findings. After admission, his temperature rose up to 37.5°C, which continued till May 13, but the next day his temperature returned to normal, never to rise again. On May 11 at night twice and on May 13 in the morning once, he had generalized clonic convulsions, but thereafter he had no convulsions. At the time of admission, he was lethargic, but on the next day his consciousness recovered slightly, and on May 15 he became quite conscious. On May 14, he vomited once. He

was able to toddle on May 15, and on May 25, he walked quite normally. During admission he complained of headache sometimes. He was discharged on July 6, but he had occasionally convulsive fits even later.

Laboratory findings. As for cerebrospinal fluid, at the time of admission, pressure was, as is given in Table I, high; fluid was clear, but

TABLE I

Cerebrospinal Fluid Findings of My Own Patient of Meningoencephalitis due to Influenza Virus B

Date	May 11	May 13	May 24	June 6
Initial pressure	280 mm	120 mm	190 mm	130 mm
Ground glass appearance	+	+	−	−
Pandy's reaction	+	+	−	−
Cell count	29/3	168/3	8/3	5/3
Sugar level	80 mg/dl	—	−	−

slight pleocytosis was seen. No bacterium was found. On May 11, red cell count was 4, 110,000 per cmm, hemoglobin 80% after Sahli's method and white cell count was 4,300 per cmm. Neutrophiles were 55.5%. Red sedimentation rate was 13 mm. on May 11, 70 mm. on May 19 and 6 mm. on June 2 in the first hour. Tuberculin test was negative. Urine was normal except that urobilinogen reaction was slightly positive on May 11, though it became negative thereafter. Hemagglutination-inhibition test for influenza virus, as is given in Table II, showed a significant rise of antibody titer for influenza virus B.

TABLE II

Hemagglutination-inhibition Titers of Serum of My Own Patient of Meningoencephalitis due to Influenza Virus B in Acute and Reconvalescent Stages

Virus ↓ Date→	May 11	May 24
Influenza A	16,384	32,768
Influenza A-prime	8,192	8,192
Influenza B	4,096	32,768

Comment

In the epidemic of Asian influenza many neurological complications were reported. But as to the cases with neurological complications in which virus infection was identified serologically or virus itself isolated, it was Brown *et al.*[2] that reported for the first time a case of influenza

complicating encephalitis. Wolff[4] reported a case of the neurological disorders due to influenza virus B with serological confirmation. Jennings[3] reported 4 cases of disorder of the nervous system due to influenza virus A which was confirmed serologically. Since 1954 outbreaks of aseptic meningitis have been seen mainly in summer every year in Aomori City and its neighborhood. From the cerebrospinal fluid, blood and throat washings of patients with aseptic meningitis viruses were isolated by us.[12] This kind of virus was identified as HVJ (Hemagglutinating Virus of Japan) or influenza D. Independently of outbreaks of aseptic meningitis caused by HVJ, influenza A was prevalent in Aomori City from the end of April to May, 1955. The present case was confirmed as meningoencephalitis due to influenza virus B by hemagglutination-inhibition test. Autopsy findings of encephalitis due to influenza virus revealed histologically acute hemorrhagic leucoencephalitis.[7] It is not difficult to imagine that the convulsive fits the present case occasionally had was due to hemorrhagic lesions in the brain caused by viral infection.

Summary

A case of meningoencephalitis due to influenza virus B is described. The diagnosis of influenza was supported by hemagglutination-inhibition test.

References

1) Leichtenstein, Dtsch. med. Wschr., 1892, **18**, 32.
2) Brown, Muether, Pinkerton & Geiger, J. Lab. & Clin. Med., 1945, **30**, 392.
3) Jennings, Brit. M. J. 1952, **1**, 123.
4) Wolff, cit. Bergmann, Frey & Schwiegk's Handbuch d. inn. Med., erster Band, erster Teil. s. 418, vierte Auflage, Springer-Verlag, 1952.
5) Dunbar, Janneson, Larglands & Smith, Brit. M. J., 1958, **1**, 1913.
6) Dubonitz, Lancet, 1958, **1**, 140.
7) Goodbody & McGill, *ibid.*, 1958, **1**, 156.
8) Flewett & Hoult, *ibid.*, 1958, **2**, 11.
9) McConkey & Daws, *ibid.*, 1958, **2**, 15.
10) Bental, *ibid.*, 1958, **2**, 18.
11) Horner, New England J. Med., 1958, **258**, 983.
12) Nakao & Yamada, Tohoku J. Exp. Med., 1959, **71**, 61.

The Tohoku Journal of Experimental Medicine, Vol. 71, No. 4, 1960

On the Nature of the So-Called Osteoclasts

By

Mizu Kojima and **Masayuki Ogata**
(小 島 瑞) (緒 方 誠 之)

From the Pathological Laboratory of Prof. K. Akazaki,
Faculty of Medicine, Tohoku University, Sendai

(Received for publication, November 19, 1959)

The multinucleated giant cell closely related with the bone tissue, first pointed out by Robin (1849), were called by Kölliker Ostoklast (the osteoclast), since he regarded these as having the function of destroying and resorbing bone tissue, and since then few, if any, author has objected to these name and function assigned by Kölliker to the cells. It can be said, however, that the positive proof of such a function has not yet been produced, except the bare fact that such cells are frequently seen present at the resorptive process of bone tissues, seemingly busy in phagocytosis thereof, under a microscope. So, the present authors attempted a rescrutiny of the question whether such cells actually deserve the name of osteoclasts, and by the way, conducted a thorough discussion of the nature of these cells.

1. Osteoclasts and Reticulo-Endothelial Cells

Considering the function of absorbing bone substance attributed to the osteoclasts, the relationship between these cells and the reticuloendothelial system (RES) has been long under attention and many an author has seen foreign body giant-cells originated from histiocytes in them (Pommer, Mallory, Haythorn, Hayashi, Hancox), but the authors have often confessed the difficulty of clearly demonstrating the presence of foreign bodies, in particular, of what can be identified as osseous matter, in such giant-cells in paraffin sections. Shipley & Macklin have denied the phagocytic ability and the participation in the RES of these cells considering their negative results obtained in their experiments of vital staining with trypan-blue and McLean & Bloom arrived at similar conclusions through observation of the osteolytic process in experimental hyperparathroidism.

For determining whether the RE cells take any part in the bone resorption process, the present authors first conducted the following experiments:

1) Intrasubcutaneous introduction of bone powder: Dried bone

powder of rabbit suspended in 0.5 cc. each of carbowax was introduced into the dorsal subcutaneous tissue of rabbits at more than a dozen spots, and the tissue was cut out spot by spot at stated intervals in the 8 following weeks; stretched preparations and cut sections were made and the resorption of the introduced bone powder was followed up.

In the subcutaneous tissue examined, at first pseudoeosinophilic leucocytes and then gradually histiocytes began to proliferate in reaction, densely besieging the group of bone particles in the first week and filling up all the spaces in the group in the third week, manifesting active phagocytosis. In about a week after the introduction of bone powder, multinucleated giant cells with essentially the same cellular nature as the histiocytes begin to appear simultaneously with the hyperplasia of the latter, then gradually increase in number and attaching themselves to many bone particles, show active phagocytosis as do the histiocytes. (See Fig. 2) These giant cells contain rounded pycnotic nuclei several scores in number per cell and bone particles in varried stage of disposing process. With the progress of disposal of the introduced bone powder, however, these histiocytes and giant cells also begin to lose in their reactive multiplying power, while the already proliferated ones begin to degenerate and to break down, so that in the 5th–6th weeks both the bone particles and the reactive cells are found decreased, the hitiocytes having mostly changed into foam-cells and karyolysis and vacuolation of the cell bodies being obvious in the giant cells. By the eighth week, when the bone powder has been nearly all resorbed up, the giant cells have become scarcely observable and the histiocytes very scanty, so that the morbid area has returned to the normal subcutaneous tissue.

2) Introduction of bone powder into bone-marrow and vital staining

Holes 2 mm. in diameter were drilled in the shaft cortex of the femoral bones of rabbits and through them dried bone powder was introduced for histological examination of the disposal process of the introduced bone powder for 8 weeks thereafter. Before sacrificing the animals, vital staining with 6 cc. $\times 6$ of 2% trypan blue solution was applied. The introduced bone powder was disposed of by the RE cells and foreign-body giant cells enormously hyperplasied reactively in the bone marrow, quite in the same manner as in the subcutaneous tissue above. (See Fig. 1) The forthcoming giant cells manifest active phagocytosis and usually show positive vital staining. These giant cells are nothing different from the other foreign-body giant cells in morphology and their fate is also similar to the simultaneously proliferated RE cells, disappearing slowly from the inflammatory foci with the completion of bone powder disposal.

On the other hand, a newly formed layer of osteoid appeared on the 14th day in the perforated part of the cortex of the shaft and here some

polynuclear giant cells with about a decade of nuclei come forth in close vicinity of the layer. These are the cells that can be identified with the osteoclasts, have spheroid nuclei, with lose nuclear nets, dark cell bodies of some thickness and are mostly connected with the osteoblasts appearing in the same places. The callus formation in the site of drilled holes become more perceptible in the 4th and 5th weeks, when not a small number of the above multinucleated giant cells are in appearance admixed with numerous osteoblasts around the newly formed callus. These giant cells are entirely negative to vital staining or as the osseous ground substance stain only very slightly light-blue and quite unlike the foreign-body giant-cells in the bone-marrow above are entirely lacking in the picture of having taken in granules of vital dye.

The present authors also studied the actual disposal process of bone substance of RE cells in various human cases. The results showed that the disposal of osseous substance by RE cells occures only in the cases where the injury in the osseous tissue leads to necrosis of bone cells and formation of sequesters, as in the cases of tuberculous osteomyelitis. The RE cells in such cases dispose of the sequesters by phagocytosis, as in the above experiments of bone-powder introduction. It need not be reiterated here that the foreign-body giant cells play a leading part in such a disposal process.

In summary of the above results, we may say that the appearance of the RES-derived multinucleated giant cells is always and necessarily preceded by an appearance of sequesters, that is, tissues of bone structure containing dead bone cells; in such cases, the giant cells contact the sequester fragments and dispose of them by phagocytosis. When, however, the tissue entirely of dead osseous substance is liquefied, a reactive proliferation of mononuclear RE cells may occur but no giant cells some into appearance. Thus, the so-called osteoclasts show difference from the foreign-body giant cells in their conditions and sites of occurrence and their fundamental cellular nature.

2. Mechanism of Bone Resorption and Osteoclasts

The above findings showing that no function of either destroying and disposing of bone tissues or liquefying them and then absorbing the liquid can be expected in osteoclasts, suggest the high improbability of the cells being active participants in the process of osseous resorption. Then, why do such cells preferentially come forth in the bone tissues that are in the process of resorption?

When rabbits are repeatedly subjected to intravenous injection of trypan blue, the RE cells in their bone-marrows are selectively stained out and an active osteolytic process often takes place. Therefore, the osseous specimens of such rabbits are well-suited for observation of the resorption

process of bones. In looking through the course of bone resorption in such cases, we find that the flat bone cells regularly arranged and mutually connected lattice-wise in the bone trabeculae first begin to sweel up and with the progress of bone resorption, the intercellular bonds become noticeable and the protoplasmic processes grow thicker. With further process of osteolysis, several osteocytes each aggregate into syncytia and in the places where the bone tissue has already disappeared these begin to turn into giant cells, which are still interconnected through processes. (See Fig. 5) Upon further progress of osteolysis the inter-connecting processes fade away, the cell bodies become smooth-surfaced and the syncytial formations grow into full-fledged giant cells. (See Fig. 8) The giant cells thus produced are always within the layer of the osteoblasts surrounding the existing bone tissue and are often connected with the latter cells through cellular processes.

When bone resorption is wide-scoped in occurrence, a space surrounded by a layer of osteoblasts is created and giant cells of varying number are left in it. (See Fig. 6, 7) Some of these cells are still in connection with osteoblasts. Frequently the layer of osteoblasts resembling a vascular endothelium is mistaken for the wall of a dilated capillary, and especially as erythrocytes left in the interspace by hemorrhage are often observed, the assumption of looking upon the finding as a ground for advocating the theory of endothelial origination of osteoclast is dangerously near. The giant cells remaining in the space soon disappear by degeneration and the space turns into a cyst. Such a process of bone resporption and the appearance of osteoclasts associated with the process may be very frequently observed in the osseous foci in Recklinghausen's disease. A part of the numerous giant cells coming forth in such foci, especially those existing group-wise in the interstices of the tissues, may be assumed to have been produced by such a process (See Fig. 3); bone fragments in the course of degenerating away are often found in such foci.

Osteoclasts, however, do not necessarily come forth wherever resorption of bone tissue is in progress. We have observed in 12 cases of multiple myeloma that scarcely any osteoclasts appeared in the site of bone resorption in these cases except where pathologic fracture was present or surgical treatment had been applied. The traveculae invaded by the tumor tissue scarcely react thereto but liquefy and disappear by themselves and the barely remaining fibrous-net is either taken into the tumor tissue or all the bone tissue is resorbed up to form a cyst. Meanwhile, the bone cells in the osseous tissue also degenerate and disappear abreast with the resorption of the intercellular substance. Accordingly, it is very rare that any cells should remain in the space where the osseous matter has absorbed and turn into giant cells.

3. Mechanism of Bone Formation and Osteoclasts

In the median sagittal sections of whole 15th day fetus of rabbits, the bone system is represented by a system of swollen cartilage cells in any part except the upper and the lower jaws, where the osteoblasts are enormously proliferated and fine arboraceous fibrous osteoids are formed amongst them. Multinucleated giant cells are already found in this stage among the osteoid tissues. These are always large-sized and are surrounded by groups of osteoblasts, with which they are connected through cytoplasmic processes, and are arranged as if in preparation of forming osteoids.

Such findings are also seen in the sites of reactive new bone formation in various bone lesions, particularly in the sites where osteoids are in formation. In any case, such are appearance of multinucleated giant cells is observed in sites where osteoblasts are plentifully proliferated but the intercellular collagenous substance is scanty, that is, where the hyperplasia in fibres cannot come up with the proliferation of osteoblasts.

Thus the appearance of multinucleated giant cells at the time of new formation of bones is closely related with proliferation of osteoblasts, so that the giant cells fade away with the disappearance of the osteoblasts. In this connection, we made the following experiments.

Rabbits were intravenously injected with 1% Evans-blue saline solution, 8 times over every other day. The results showed that while in the untreated control animal, numerous osteoblasts and osteoclasts were found scattered about in the metaphysis of Femur, in the treated animals, the cuboidal basophilic osteoblasts were away as if really wiped off and also the osteoclasts were entirely absent. The bone trabeculae were also much atrophied and the osteoplastic process was very poorly developed in the tested animal, as compared with those in the control.

The occurrence of osteoclasts was especially frequent in the cases where osteoid tissues come into formation, of all the cases of new bone-formation. The progress of endochondral ossification was observed in rabbits 80 days after birth and about 1,500 g. in body weight after intramuscular injection of 5 mg./kg. each day and 150 mg. in total of DOCA and it was found that the delay in degeneration of cartilage cells and the overproduction of osteoid tissues were very perceptible, while more multinuclear giant cells were found commingled with the numerous basophilic osteoblasts making up such osteoid tissues in the femur, than in that of the untreated control. The newly formed osteoid tissues partly ossify, but the remainder mollify, both their cellular and fibrous components disappear and cysts are formed in the place. Such formations of osteoids poor in the tendency to ossification and of cysts by secondary degeneration are most noticeable in the cases of the so-called bone cysts. In these cases

too, many giant cells appear accompanying the proliferation of osteoblasts and often many giant cells are found grouped around the cysts formed by secondary degeneration. These giant cells in group are understood to have been formed by syncytial aggregation of many cell components left back by the disappearance of fibrous components of osteoids. If, however, the hyperplasy of fibres goes well abreast with the proliferation of osteoblasts, multinuclear giant cells rarely come forth, even in the cases of osteoid formation. Such a finding is frequent in the foci of fibrous dysplasia.

Discussion

The bone tissue is composed of cellular components and of an intercellular substance consisting of collagenous fibres, cement substances and bone salts. In the mechanism of resorption of a bone tissue, the above constituents do not necessarily go down at the same pace. When the cellular components first die away, the tissue falls into necrosis and changes into a sequester, which is disposed of by absorption by the RE cells, acting upon it in the same manner as if the sequester was a common foreign-body. On such an occasion, the multinucleated foreign-body giant cells make appearance and manifest active phagocytosis. The RES comes into the play in such cases only. But in the cases where the cellular components are comparatively intact but the intercellular substance alone is dissolved and resorbed, the lattice-like reticular structure of the bone cells in normal state turns spongy owing to the dissolution of the substance that has filled up the intercellular spaces and then form syncytia which finally take the form of giant cells. The third process of bone resorption is present when the cellular components degenerate and break down together with the dissolution of intercellular substance. Here, a vacant space is left behind by the disappearing bone. In the second type process above too, when the residual giant cells degenerate and pass away, a similar vacancy is created. The above processes of bone extinction may be summarized schematically as follows:

1. Necrosis of bone cells—Sequester—Disposal by histiocytes (Appearance of foreign-body giant cells)
2. Dissolution of intercellular substance—cellular osteoid (Appearance of bone giant-cells)
3. Dissolution of intercellular substance + necrosis of bone cells—Formation of vacant space (Cyst)

At new formation of a bone tissue, the above bone constituents do not always increase at the same pace, and by such an unbalance in the ossification process, various spurious bone tissues come into formation. Even when the proliferation of osteoblast and the increase of fibrous components go on at normal pace, if the deposition of bone salts falls arrear, the formation will be only a fibrous bone-like tissue (fibrous osteoid) instead of a

bone tissue. We can be hardly expect a multinuclear giant cell to appear in such a tissue. Next, when the osteoblasts alone are multiplied in an attempt to form a bone tissue but the component fibres fail to increase abreast, the osteoblasts cannot take their proper reticular structure and in the place of a bone tissue, syncytial groups of bone cells or multinucleated giant cells make appearance. Such a formation process of giant cells may be regarded as essentially similar to that of bone resorption described above. From such a ground, the giant cells formed under such a circumstance may be taken to represent, so to speak, components of bone tissue lacking in fibrous components and bone salt. Accordingly, we may perhaps call such giant cells so many ' cellular osteoids.'

Summary

Upon examination of the multinucleated giant cells closely related with bone tissues from various angles, it was demonstrated that the so-called osteoclasts are nothing but osteoblastic cells taking syncytial form at the stages of dissolution or new formation of trabeculae and cannot be regarded as having the function of positively destroying and resorbing bone substance. Accordingly, we deem it unjustifiable to call them osteoclasts and propose the new name of ' bone giant cells ' for them.

References

1) Arey, Anat. Rec., 1916–17, **11**, 319., Anat. Rec., 1917, **13**, 269, & Amer. J. Anat., 1919, **26**, 315.
2) Askanazy, Zbl. Path., 1902, **13**, 369.
3) Barnicot, 3 Proc. R. Soc. B., 1947, **134**, 467.
4) Bloom, W. & Bloom, M. A., Anat. Rec., 1940, **78**, 497.
5) Cobb, Arch. Path., 1953, **55**, 496.
6) Dontenwill, Ziegler's Beitr., 1957, **117**, 50.
7) Duthie & Barker, J. Bone Surg., 1955, **37**B, 691.
8) Greep, J. Amer. Dent. Ass., 1948, **36**, 427.
9) Häggqvist, Acta Chir. Scand., 1928, **65**, 180.
10) Ham, J. Bone Surg., 1930, **12**, 827.
11) Hancox, J. Physiol., 1946, **105**, 66, & Biol. Rev., 1949, **24**, 448.
12) Hayashi, Chügai Iji Shimpô, 1915, **515**, 859.
13) Heller, McLean & Bloom, Amer. J. Anat., 1950, **87**, 315.
14) Heller-Steinberg, Amer. J. Anat., 1951, **89**, 347.
15) Haythorn, Arch. Path., 1928, **7**, 651.
16) Jaffe, Arch. Path., 1929, **8**, 817, Arch. Path., 1933, **16**, 63.
17) Jordan, Amer. J. Anat., 1918, **24**, 225.
18) Kirby-Smith, Amer. J. Anat., 1933, **53**, 377.
19) Kölliker, Gewebelehle des Menschen, Bd. I. Verlag v. W. Engelmann, 1889.
20) Kojima, Takagaki, Watanuki, Ogata, Kuroda & Oota, Tohoku J. Exp. Med., 1958, **68**, 207.
21) Kroon, Acta anat. Basel., 1954, **21**, 1.

22) Maximow & Bloom, Textbook of Histology 6 Ed. W. B. Saunder, 1953.
23) McLean & Bloom, Anat. Rec., 1940, **78**, 333, Arch. Path., 1941, **32**, 315.
24) Meyer, J. Path. & Bact., 1956, **71**, 325.
25) Niven & Robison, Biochem. J., 1934, **28**, 2237.
26) Petersen, Histologie u. mikroskopische Anatomie, Verlag v. J. F. Bergmann.
27) Pommer, Virchow Arch., 1883, **92**, 276.
28) Pommer, Zbl. Anat., 1925, **75**, 382.
29) Prentis, Surg. Gynec. Obst., 1952, **20**, 670.
30) Robinson, J. Bone Surg., 1952, **34**A, 389.
31) Sandison, Amer. J. Anat., 1928, **41**, 447.
32) Shipley & Macklin, Anat. Rec., 1916, **10**, 597, Amer. J. Physiol., 1917, **42**, 117.
33) Sissons, Brit. J. Surg., 1951, **39**, 172.
34) Sundberg & Hodgson, Rev. Berge Path., 1955, **24**, 251.
35) Tichendorf, Klin. Wschr., 1956, **28**, 21.
36) Urist, J. Bone Surg., 1941, **23**, 1.
37) Weidenreich, Handb. d. mikroskop. Anat. d. Mensch., II/2, W. v. Möllendorff, Springer, Berlin, 1930.
38) Weinmann & Sicher, Oral Surg., Oral Path. & Oral Med., 1955, **8**, 1074.
39) Weinmann, J. Bone Surg., 1956, **38**, 1335.

Fig. 1

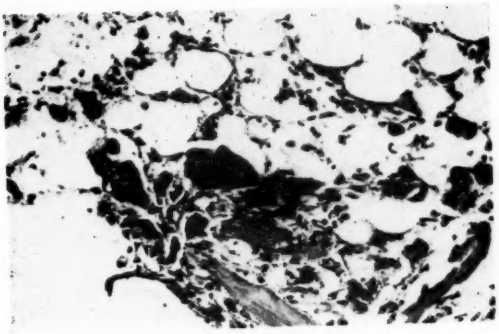

Fig. 2

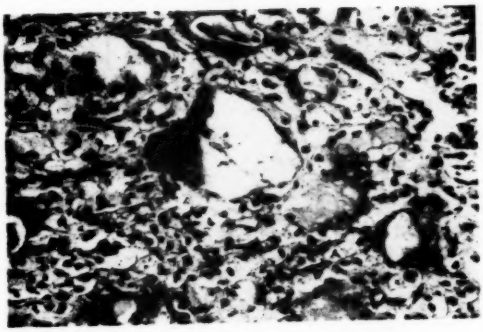

Fig. 3

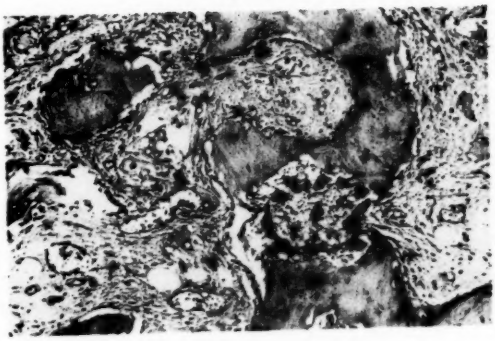

Fig. 4

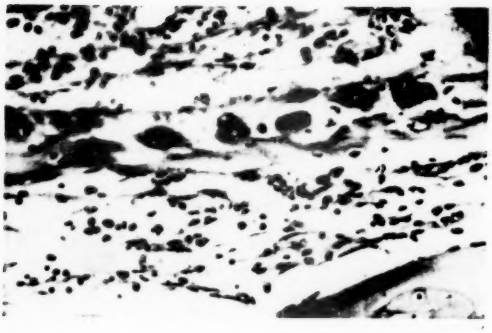

Fig. 5

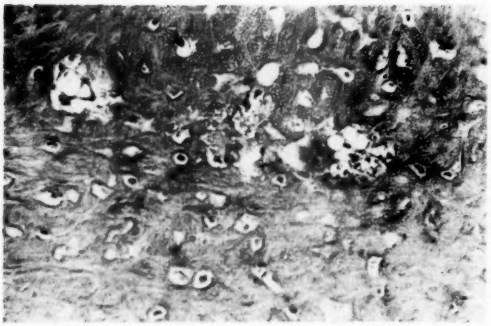

Fig. 6

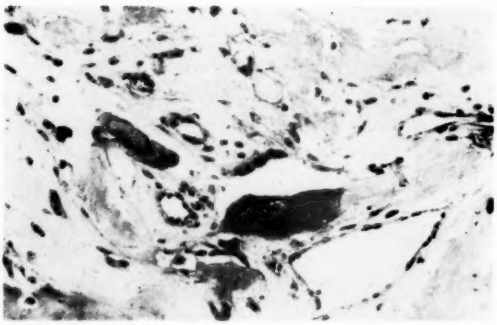

Fig. 7

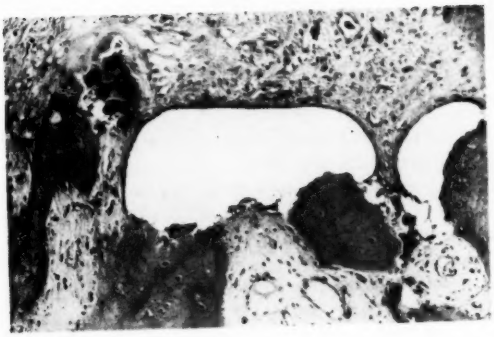

Fig. 8

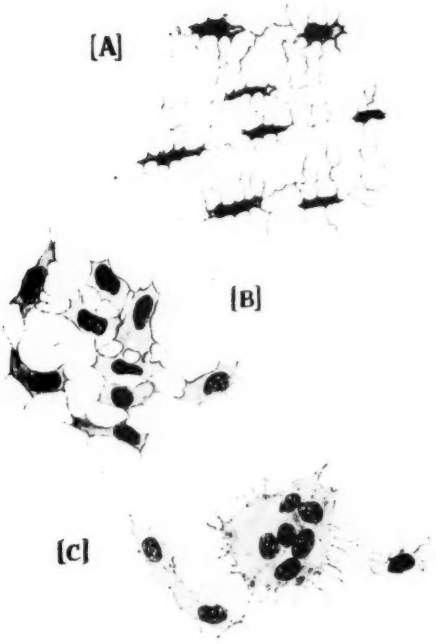

Fig. 1. Bone-marrow 21 days after insertion of bone powder: Numerous histiocytes and foreign-body giant cells aggregated around the introduced bone powder particles. The bodies of these phagocytic giant cells are full of bone powders. (Masson's staining.)

Fig. 2. Subcutaneous tissue 7 weeks after insertion of bone powder: The bone powder has been almost all resorbed and the foreign body giant cells covering it show marked degeneration by pycnosis or swelling. (H.-E. staining.)

Fig. 3. A bone in the course of resorption in Recklinghausen's disease: Numerous osteoclasts seen in the places of the trabeculae annihilated by resorption. These are in contact with the layers of osteoblasts. (H.-E. staining.)

Fig. 4. A rabbit bone vital stained with 11 doses of 5 cc. of 2% trypan blue: Showing the same findings as the preceding. (H.-E. Staining.)

Fig. 5. Femoral bone of a normal rabbit 100 days after birth: At the central part of the greatly hypertrophied trabecula, the cell lacunae are widened, the osteocytes are swollen and then fused together and osteoclast come into formation. (H.-E. staining.)

Fig. 6. Fig. 7. Myositis ossificans: Cystic spaces are formed in the vacancies left by the resorbed trabeculae, and osteoclasts form a part of the cystic walls. (H.-E. staining.)

Fig. 8. A: The osteocytes in the trabecula are connected through processes and form a lattice-like reticular structure.

B: With the dissolving away of the intercellular substances (collagen, bone salts) the hypertrophied bone cells aggregate and fuse together by shortening of the connecting processes.

C: By such a process the multinucleated giant cells come into formation. These are what have been called the osteoclasts hitherto.

The Tohoku Journal of Experimental Medicine, Vol. 71, No. 4, 1960

Studies on Giant Cell Tumors of Bone

On the Nature of the Giant Cells and the Malignity of the Tumor

By

Mizu Kojima and **Masayuki Ogata**
(小島　瑞)　　(緒方　誠之)

From the Pathological Laboratory of Prof. K. Akazaki,
Tohoku University School of Medicine, Sendai, Japan

(Received for publication, November 19, 1959)

The histological characteristic of the giant-cell tumor of the bone is that it is accompanied by the appearance of a vast number of multinucleated cells. The essential nature of the tumor has been studied and the annals of the study is already long, for it begins since Nelaton assumed these multinucleated giant cells as one of the constitutional elements of the tumor tissue, but still the question remains unsolved. Although it is the most prevalent opinion to regard the tumor to be osteogenic in origin, seeing that the multinucleated giant cells which characterize this tumor bear a close resemblance to osteoclast (Kölliker, 1873), the character of these osteoclasts themselves is yet unclearified, so that no definite pathological answer is forthcoming to the question of how much we should evaluate the malignity of this tumor or whether the tumor cells are capable of forming bone tissue. The present authors have reported on their study on the so-called osteoclast in the preceding paper, in which the following conclusion was arrived at: The giant cells in the question are formed through the osteolytic process by the liberation and aggregation of the bone cells following the disappearance of the intercellular substance (bone salt, collagenous fibres etc.); when only a proliferation of the osteoblasts has occured first and has not been followed by new formation of the intercellular substances in bone-forming process, the cellular components tending toward forming bone trabeculae often aggregate and fuse together; thus, the multinucleated giant cells are also formed. The multinucleated giant cells formed through such processes are generally called the osteoclast regardless of their production process, either by osteolysis or by new bone formation, but these giant cells, as is evident from the mechanism of their formation, have no bone-destructive or resorptive function, as the name would suggest, but rather constitute the osteoid tissue that consists of the cellular components alone destitude of fibres and bone salts, so that the giant cells should be regarded as so many as

cellular osteoid tissue in their essential nature.

Basing ourselves on such findings, we now proceeded to examine specimens from 29 cases of giant-cell tumors of bone, with specimens from 42 cases of osteosarcomata and bone cysts etc. as controls (Table II), laying stress on the form of the multinucleated giant-cells and the relation-

TABLE
Clinical

No. pat.	Age/Sex	Injury	Duration	Location(end)
1 T.K.	30/W	(−)	5 mos.	Tibia upper
2 T.O.	33/W	(+)	8 mos.	Tibia upper
3 T.S.	24/W	(−)	9 mos.	Femur lower
4 T.B.	29/W	(−)	2 yrs.	XI. B. vertebrae
5 N.K.	/W	(−)	6 mos.	Tibia upper
6 K.W.	/M	(−)	——	IV. B. vertebrae
7 K.O.	32/M	(−)	——	II. B. vertebrae
8 M.S.	60/M	(−)	3 mos.	fibula upper
9 M.C.	16/M	(+)	8 mos.	Humerus upper
10 M.H.	29/W	(−)	14 mos.	Humerus upper
11 T.A.	31/M	(−)	3 yrs.	Tibia upper
12 S.	56/W	(+)?	2 mos.	Tibia upper
13 K.S.	29/W	(−)	3 yrs.	Tibia upper
14 M.T.	27/W	——	——	Femur lower
15 M.Y.	30/W	(+)	4 mos.	Radius
16 H.S.	28/W	——	3 yrs.	Sacrum
17 T.K.	36/M	——	7 mos.	Femur lower
18 M.T.	36/W	——	6 mos.	fibula upper
19 M.S.	17/W	——	6 mos.	Clavicle
20 N.M.	28/M	——	3 yrs.	Tibia upper
21 H.S.	23/W	(−)	5 mos.	Tibia upper
22 S.K.	47/M	——	6 mos.	Femur lower
23 C.G.	16/W	(−)	2 mos.	Tibia upper
24 M.O.	35/W	(+)	4 mos.	Tibia upper
25 G.T.	39/M	——	6 mos.	Radius, ulna
26 E.Y.	69/M	(−)	5 mos.	Femur lower
27 A.S.	30/M	(−)	——	Tibia upper
28 Y.K.	20/W	(+)	3 yrs.	Patella
29 K.	39/M	——	5 mos.	Femur lower

ship between the distribution of the intercellular fibres and that of the giant cells. The specimens were decalcified with 5% nitric acid, cut into paraffin sections and stained with H-E staining, with PAS staining (Lillie) and with variously fibre staining such as Masson-Goldner's, van Gieson's, Mallory-Azan and Pap's silver impregnation method, etc.

I

Features

Symptoms	Treatment	Results
pain	curretage, 5 mos. later amputation	well 2 yrs. & 3 mos.
pain	amputation	well 1 yrs. & 8 mos.
pain	curretage, 15 mos. later amputation	lung metastasis 3 mos. after amput.
pain	excision	well 2 yrs. & 9 mos.
tumor, pain	3 curretage, amputation	well 2 yrs.
paresthesia	not treated	dead 2 yrs. later
pain	curretage	well 8 mos.
pain	——	——
pain, tumor	excision, amputation	metastases 9 mos. after amputation, dead 32 mos. later
pain	excision	well 4 yrs.
tumor, pain	roentgen ray, amputation	well 2 yrs. & 4 mos.
tumor, pain	amputation roentgen rays	lung metastasis 1 mo. after amputation
pain	curretage	well 3 yrs.
——	amputation	well 5 yrs.
tumor	curretage	well 2 yrs.
pain	curretage roentgen rays	well 5 yrs.
pain	curretage	well 5.5 yrs.
swelling	excision	recurrence 33 mos. after op. alive well
pain, tumor	excision	well 5 yrs. & 4 mos.
dysfunction	amputation	well 4 yrs. & 3 mos.
tumor, pain	curretage, roentgen rays, amputation	recurrence 21 mos. after op., well 2.5 yrs. after amputation
dysfunction, pain	curretage	well 3 yrs. & 4 mos.
pain	amputation	discharged well
swelling, pain	curretage	well 3 yrs.
pain	excision	lost
dysfunction, pain	amputation	alive well
pain	curretage	well 2 yrs.
tumor, pain	excision	discharged well
tumor, pain	curretage	discharged well

TABLE II

Tumors and Lesions of Bone as The Control

1)	Malignant Tumors	
	Osteogenic sarcomas	5
	Polymorphous cell sarcomas	3
	Osteochondrosarcomas	4
	Spindle-cell sarcoma	1
	Fibrosarcoma	1
	Chondrosarcoma	2
2)	Bone Cysts	7
3)	Recklinghausen's diseases	2
4)	Fibrous dysplasia	1
5)	Non-osteogenic fibroma	1
6)	Aneurysmal bone cysts	2
7)	Giant-cell epulis	1
8)	Multiple myelomas	8
9)	Osteomyelitis (include tuberculous one)	4

Results

(1) Analysis of Giant-Cell Tumor Cases

1. Clinical Findings: The clinical findings were as summarized in Tab. I. The mean age of first occurrence of the disease was 32.9, the cases in the thirties numbering 11, the highest number under this heading. Of those in the second decade, 2 were 16 and 1 was 17. 11 were male and 18 female cases. Of the sites of the tumors, the tumor occured at the upper end of the tibia in 11 cases (37.9%), at the lower end of the femur in 6 cases (20.7%) and the tumor in these two regions accounted for the majority of the total cases. Of the total 29 cases, 12 were treated by curettage and bone-transplantation (with X-ray treatment in combination), in 7 cases the tumor was resected and in 12 cases amputation or disarticulation had to be applied, while one was treated in some other way and one was left untreated. The late results were clinically or histologically evaluated as follows: The tumor was found advanced in malignity more than at the first onset of the disease in one case, post-operatively recrudesced in 7 cases and metastasized to the lung owing to malignant degeneration in other 3 cases.

2. Histological Findings: In every cases, the tumor was composed chiefly of multinucleated giant cells and spindle-, ovoid- or polygonal-shaped mononuclear cells, which were connected with one another through their fine processes, forming a reticular structure in general appearance. Erythrocytes were often found in the thinly spread intercellular meshes,

suggesting the large blood content of the tumor. No other hemaginomatous structure, however, could be found anywhere.

a) Appearance of tumor giant cells and hyperplasia of fibrous stroma: The specimens used in this study showed too manifold general and local differences in their mode of fibrous proliferation for generalization. In Case No. 2, amputated on account of advanced destruction of the bone cortex, the tumor was a typical giant-cell tumor of bone containing numerous giant cells. Under Masson's staining, some parts of the specimen were found containing only red-brown staining cellular components in dense aggregation but no fibre at all, but in other parts, a few struggling light-green staining fibres were found among the cells, while in some other parts, tumor giant cells and mononucleated cells could be seen only very sporadically in the green-blue stroma rich in fibres. As the formation of the fibres becomes the more active, the cells become the more sporadic in exsistence and finally disappear altogether. Such findings were cofirmed not only in the periphery of the tumor or in the hemorrhagic foci, but everywhere inside the tumor cordalwise or sporadically. In Case No. 23, only a few fine argyrophil fibres were found running through the parts where the multinucleated giant cells were particularly predominant and the mononucleated cells were scanty and the border between the cell-bodies of adjoining giant-cells was blurred. In proportion to the number of the mononucleated cells in exsistence, the formation of argyrophil fibres become more active and frequently arborescent thick fibres and tumor cells entagled therein fill up the spaces among the fibres. When the collagenous fibres increase in number, the giant cells decrease more and more and their cell bodies give the impression of tenuity, both their nuclei and cytoplasm being pycnotized and dwarfed, and the arrengement of the cells becomes exceedingly sporadic. In Cases No. 3 and No. 5, the fibrous proliferation was found in some circumscribed parts in the tumor and at the central part of such fibrosis, findings suggestive of osteoid formation through hyalinization were observed. In Cases No. 7, No. 11 and No. 24, callus was found formed besides the fibrous osteoid. From such findings, it can be deduced that the tumor cells in giant-cell tumor of bone evidently have a faculty to form bones, and the tumor must be a sort of neoplasm produced by osteoblastic cells.

b) Malignity of giant cell tumor of bone and morphological changes of the tumor giant cells: In the next place, the cases that seemed to indicate a turn to clinical malignance such as recurrence and metastasis were examined, with the aim of studying the effect of the developed malignity upon the morphology of the giant cells. Case No. 24 had been treated by curettage and bone transplantation alone. The patient is progressing fevorably for 5 years since the operation. The mononucleated cells are

rich in protoplasm and regularly formed, no mitotic figure being apparent. The giant cells are medium-sized, contain 10–20 nuclei each, have acidphil cytoplasm and give the appearance of thinness. The nuclei are not uniform, some being large and light, others small and pycnotic, but never anaplasied. Case No. 5 was finally subjected to amputation of the leg after curettage applied three times. In the curettage specimen, the tumor cells, circular or polygonal in form and nearly uniform in size, were arrenged sporadically as if forming meshes and showed mitotic figures in some rare cases. The sporadic tumor giant cells are of markedly gigantic size and often a swarm of more than a hundred ovoid nuclei resembling those in the mononucleated cells is found in one such a giant cell, while some small-sized multinucleated cells with only 5 or 6 nuclei are also in appearance. In the amputation specimen of the same case, the cells are densely proliferated and syncytial arrangement is prominent, mitotic figures are frequent, but the giant cells are diminutive, containing a few to 10 nuclei at most. Thus, the giant cells are so reduced in size that they are only hardly distinguishable from the mononuclear tumor cells and hence it appears that the atypism of these cells and the increase of mitotic figures sufficiently evidence the rapidity of the cell proliferation in the tumor (See Fig. 1, 2). Case No. 3 was once subjected to curettage and bone transplantation, but a year afterwards the tumor recurred and its surrounding bony cortex began to show a tendency to resorption, so that amputation at the femur had to be resorted to, but metastasis to the lung occurred three months after the operation. The curettage specimen contained polymorphic tumor cells solidly proliferated, rich in chromatin and showing mitotic figures sporadically in a few cases. Mixed with these tumor cells, there were some multinucleated giant cells containing about 10 nuclei and a lesser number of giant cells with strangely lobulated nuclei, while figures of transition between the mononucleated cells and the giant cells were obvious. In the amputation specimen, the above polymorphism was even more prominent and a partial figure of a polymorphocytic tumor was apparent, tumor cells with 2 or 3 chromatin rich nuclei being abundant and anaplasia being advanced. (See Fig. 3, 4) The Case No. 9 had died, curettage and amputation at the site of primary occurrence of the tumor, the proximal epiphysis of the right humerus, proving ineffective in preventing metastasis to the lungs, the femoral bones, the subcutaneous tissue and the pubic bone. In the drill biopsy specimen, the tumor consisted of numerous multinucleated giant cells and spindle and ovoid cells frequently showing mitosis and polymorphism. In the amputation specimen, the tumor cells were mostly giant cells containing bizarre lobulated nuclei and showing frequent atypical mitosis in progress, suggesting the picture of a polymorph-cell sarcoma. The histological findings at autopsy

suggested the presence of a polymorph-cell sarcoma, spindle cells, giant cells with gigantic irregular nuclei and multinucleated giant cells being in evidence. (See Fig. 5, 6, 7) In Case No. 20, the majority of the giant cells appearing in the specimen contained irregularly lobulated nuclei, i.e., were so-called malignant giant cells. The tumor cells also frequently showed multipolar mitoses or such atypical mitotic figures, indicating the exceedingly high malignity of the tumor. The clinical course taken by the patient also amply endorsed the malignant nature of his tumor. (See Fig. 8)

As shown above, the polymorphism of the mononucleated tumor cells is intensified as the malignity of the tumor advances, cells with 2 or 3 nuclei often appearing among them; these plural nuclei are severely anaplasied and atypical, sometimes bizarre large nuclei making appearance. Such morphological changes as seen in the tumor cells come force simultaneously also in the tumor giant cells, higher frequency of atypism, decrease of nuclei and appearance of irregular lobulated nuclei becoming apparent in them, too. Such findings cause the histological picture of the giant-cell tumor of bone to pass over into that of an osteogenic polymorphous-cell sarcoma or the so-called osteolytic osteosarcoma.

Discussion

The abovestated results of examinations led the authors to conclude that though a giant cell tumor of bone is generally rich in cellular components and show poor formation of fibrous stroma, still in some parts or in some cases, the proliferation of the latter is rather active and hyalinization of the circumscribed foci of hyperplasied fibrous stroma may lead to formation of fibrous osteoid and callus, so that the mother tissue of such a tumor must be nothing but the bone tissue and that an antagonism exists between the hyperplasia of fibrous stroma and the formation of the multinucleated giant cells in the tumor. In collation of such findings with the results of our previous study on osteoclasts, it is inferred that the giant cells constituting such a tumor undoubtely correspond to the bone giant cells, especially those that appear in the osteoplastic process, and should be regarded as tumorous cellular osteoids in nature. Accordingly, now that the osteoclastic faculty of such bone giant cells has been denied, it is clear that the opinion of Stewart, Willis and some others, who roundly negated the possibility of any osteoplastic process in the tumor tissue, considering the appearance of the giant cells only in connection with the active osteoclastic tendency manifested by the giant cell tumor, is evident misstatement of the truth. If the tumor giant cells are assumed to form our so-called tumorous cellular osteoids, the appearance of circumscribed fibrous foci and fibrous osteoids in the tumor tissue may be interpreted as indicating

a tendency of the differentiation of the tumor cells facilitating formation of fibrous rather than cellular osteoids.

For interpreting the histogenesis of the giant-cell tumors of bone, at present the theory of undifferentiated mesenchymal cell origin is widely supported, besides the abovementioned theory of osteogenic origin laying importance on the osteoclasts. The former theory explains the variegated histological findings observable in such a tumor, such as the reticular arrangement of the tumor cells, the appearance of blood spaces and figures of new bone formation etc., by ascribing a totipotence of the undifferentiation of the tumor cells, but if we follow the system of classification of such cells by Jaffe and Lichtenstein, the main advocators of this theory, we must face the contradiction that while the cells of Grade I of advanced differentiation do not show any tendency to bone formation, the cells of their Grades II and III of further advanced malignity show some capacity for bone formation, and their theory does not give any definite exposition of the essential nature of the tumor giant cells. Some of the authors who identify the bone giant cells with foreign-body giant cells inconsistently deny the phagocytic ability of the tumor giant cells (Aegerter and some others), or sometimes refer to the bleeding foci in the tumor cells specifically as the foreign-bodies inciting the formation of such giant cells (Barrie). Since, the present authors have denied the formation of foreign-body giant cells to dispose of liquified necrotic foci in the paragraph on such cells in their previous study on the bone giant cells, the relationship between the foreign-body giant cells and the giant cells coming forth in a giant cell tumor of bone may be absolutely abnegated. The relationship between capillary endothelial cells and the giant cells coted as a ground for assuming a vascular-tissue origin of such a tumor (Stroebe, Mönckeberg, Johnson) can be denied if we recall the relationship between the cysts formed subsequent to osteolysis and the giant cells, as detailed in our previous report.

In the next place, we will speak on the evaluation of the malignity of such tumors. It has been already accepted that the mononucleated tumor cells fall into anaplasia with the malignant degeneration of the tumor and we may anticipate that the giant cells of the same cellular nature as these tumor cells would also show anaplasia at the same time. Indeed, cases of reduction of the giant cells in size and appearance of malignant-type giant cells with irregularly lobulated nuclei have been reported. What would be the mechanism of production of such findings?

Multinucleated giant cells assuming an organoid structure, such as of cellular osteoid, symbolize the differentiation of the subject tumors and thus essentially indicate their benignity, so that we must first rely on the behavior of the mononucleated tumor cells for evaluating the malignity of the tumors. But when the anaplasia of the tumor cells is advanced, the

organoid structure formed thereby also falls into displasia or anaplasia, so that the giant cells come to be reduced in their number of nuclei and their size and eventually to show irregularly lobulated nuclei in them. Thus, the polymorphism of the tumor cells become gradually more manifest and in highly malignant tumors multinucleated giant cells of benign type or the so-called epulis type become hardly detectable and the picture of polymorphous-cell sarcomata is shown. In this sense, we may understand why a malignant giant-cell tumor of bone and a polymorphous-cell sarcoma or an osteolytic osteosarcoma show similar histological pictures.

In the next place, we must note that there are reports on the tranformation of giant cell tumors of bone into osteoplastic osteosarcomata and fibrosarcomata upon their turning malignant. Such transformations may be explained by attributing them to the accentuation of some of the components of the bone tissue affected by the osteogenic tumor, including cells, fibres and bone salts. Giant cell tumor tissue may be found accompanying an osteogenic sarcoma tissue mainly characterized by bone formation, and such giant cell tumor tissue may appear also in benign tumors, such as osteoma and osteofibroma.

Summary

Specimens from 29 cases of giant cell tumors of bone and 42 cases of other bone tumors were pathohistologically examined, with emphasis on the relationship between the multinucleated giant cells and the fibrous stroma in them. The results were summarized as follows:

The giant cell tumor of bone is an osteogenic tumor characterized by the appearance of multinucleated giant cells constituting a cellular osteoid tissue, with the grade of malignity indicated by the behavior of the mononucleated tumor cells. With the advance of malignity, the organoid giant cells fall into hypoplasia or dysplasia, oligonucleated giant cells and those of malignant-type coming forth, and the tumor gradually takes on the histological picture of a polymorphous-cell sarcoma. Giant cell tumor tissues may be found sometimes in benign osteoma, osteofibroma and malignant osteoplastic osteosarcoma, too.

References

1) Aegerter, Amer. J. Path., 1947, **23**, 283.
2) Albertini, Gutartige Riesenzellengeschwülste, Tieme-Verlag, Leipzig, 1928, Histologische Geschwulstdiagnostik, Tieme-Verlag, Stuttgart, 1955.
3) Barrie, Ann. Surg., 1920, **71**, 581.
4) Bloodgood, Ann. Surg., 1910, **52**, 145, Surg. Gynec. Obst., 1924, **38**, 784
5) Brailsford, Lancet, 1943, **1**, 776.
6) Codman, Surg. Gynec. Obst., 1931, **52**, 543.
7) Coley & Higinbotham, J. Bone Surg., 1938, **20**, 870.

8) Coley, Amer. J. Surg., 1935, **28**, 768.
9) Compere, J. Bone Surg., 1953, **35** A, 822.
10) Cooper & Traverse, Surgical Essays, London, 1818, 75.
11) Coventry & Dahlin, J. Bone Surg., 1957, **39** A, 741.
12) Dahlin, Proc. Mayo Clin., 1956, **31**, 31.
13) Davlin, Bowman & Mitchell, J. Bone Surg., 1955, **37** A, 472.
14) Dyke, J. Path. & Bact., 1931, **34**, 259.
15) Ellis, J. Bone Surg., 1949, **31** B, 268.
16) Finch & Gleave, J. Path. & Bact., 1926, **29**, 399.
17) Geschickter & Copeland, Tumors of Bone, J. B. Lippincott Co., Philad., Ed. 3, 1949.
18) Gregory, N. Y. State J. M. 1955, 3269.
19) Haslhofer, Handb. spez. path. Anat., 1937, **9**/3, 477.
20) Hellner, Die Knochengeschwülste, IIte Auf., Springer-Verlag, Berlin, 1950.
21) Hillmanns, Beitr. Klin. Chir., 1954, **189**, 455.
22) Hueck, Ziegler's Beitr. 1920, **66**, 330.
23) Jaffe, Lichtenstein & Portis, Arch. Path., 1940, **30**, 993, Amer. J. Path., 1942, **18**, 205, Bull. N. Y. Acad. Med., 1947, **23**, 497, & Ann. R. Coll. Surgeons, 1953, **13**, 343.
24) Johnson, Arch. Path., 1930, **10**, 197.
25) King, Brit. J. Surg., 1932, **20**, 269.
26) Kleinberg, J. Bone Surg., 1939, **21**, 433.
27) Kojima & Ogata, Tohoku J. Exp. Med., 1959, **71**, —
28) Leucutia & Cook, Amer. J. Roentg., 1949, **612**, 685.
29) Levy, Amer. J. Path., 1948, **24**, 723.
30) Lichtenstein, J. Bone Surg., 1951, **33** A, 143.
31) Lubarsch, Erg. allg. Path. u. path. Anat., 1895, I/II, 364.
32) Mallory, J. Med. Res., 1911, **24**, 463.
33) McGuire & McWhorter, Arch. Surg., 1924, **9**, 545.
34) Meyerding, J. A. M. A., 1924, **83**, 1323.
35) Miller & Monteleone, Frankf. Z. Path., 1957, **68**, 49.
36) Mönckeberg, Virchow Arch., 1923, **246**, 106.
37) Morton, Caner (Philad.), 1956, **9**, 1012.
38) Murphy & Ackerman, Cancer (Philad.), 1956, **9**, 317.
39) Nelàton, D'une nouvelle espéce de tumeurs benignes des os, ou tumeurs à myeloplaxes, Paris, Adrien Delahaye, 1860.
40) Pierce & Lampe, J. A. M. A., 1936, **107**, 1867.
41) Platt, Surg. Gynec. Obst., 1935, **60**, 318.
42) Prossor, J. Bone Surg., 1949, **31** B, 241.
43) Puhl, Beitr. path. Anat., 1936-37, **98**, 335.
44) Ribbert, Frankf. Z. Path., 1917, **20**, 29.
45) Russell, J. Bone Surg., 1949, **31** B, 281.
46) Shuffstall & Gregory, Amer. J. Path., 1953, **29**, 1123.
47) Stewart, Lancet, 1922, 1106, Amer. J. Path., 1938, **14**, 515, & J. Bone Surg., 1952, **34** A, 372.
48) Stone & Ewing, Arch. Surg., 1923, **7**, 280.
49) Stroebe, Beitr. path. Anat. u. Physiol., 1890, **7**, 341.
50) Thomson & Turner-Warwick, J. Bone Surg., 1955, **37** B, 266.
51) Williams, Dahlin & Ghormley, Cancer (Philad.), 1954, **7**, 764.
52) Willis, J. Bone Surg., 1949, **31** B, 236.
53) Windeyer, J. Bone Surg., 1949, **31** B, 252.

Fig. 1

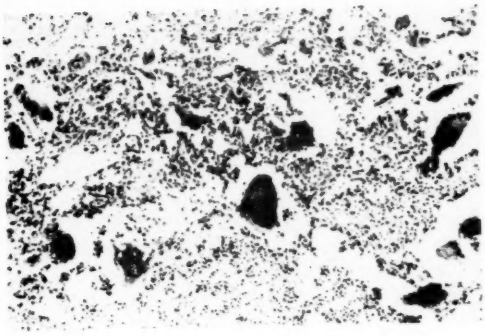

Fig. 2

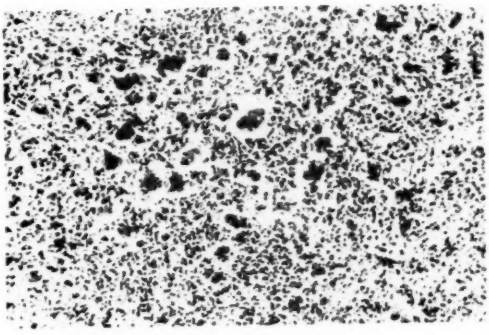

Fig. 3

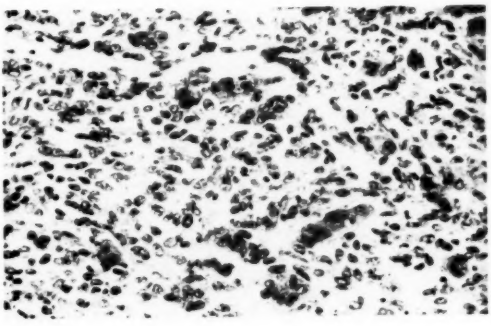

Fig. 4

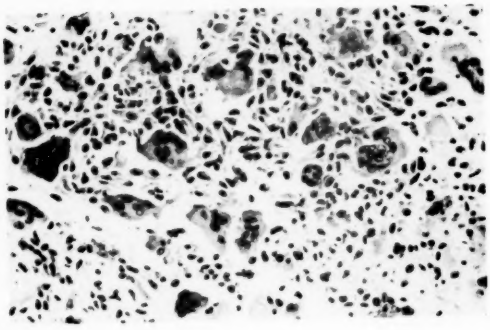

Fig. 5

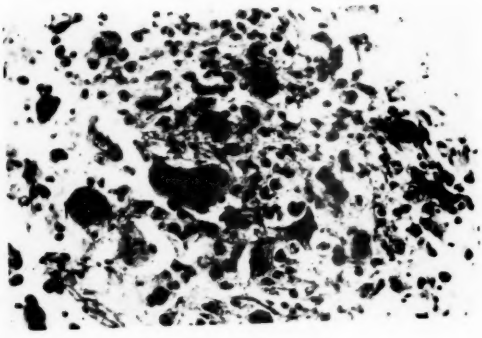

Fig. 6

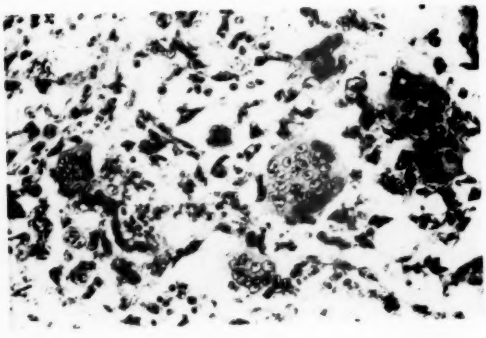

Fig. 7

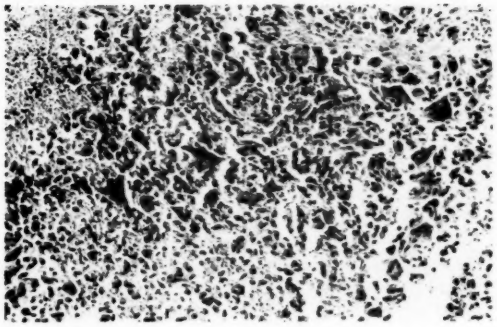

Fig. 8

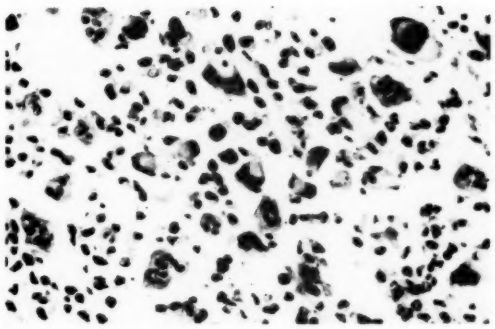

Fig. 9

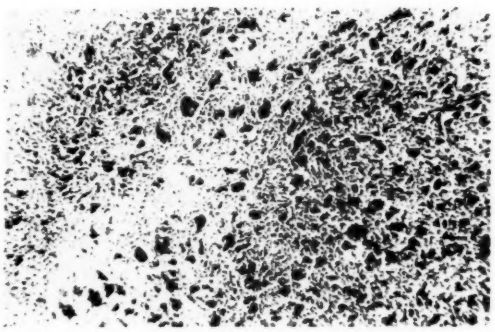

Fig. 10

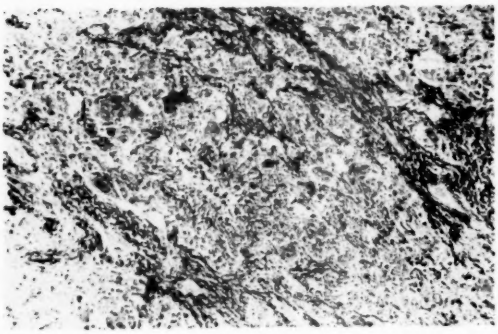

Fig. 11

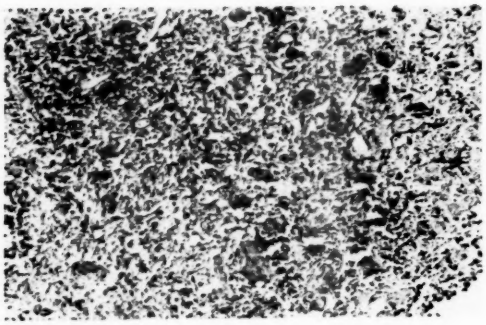

Fig. 12

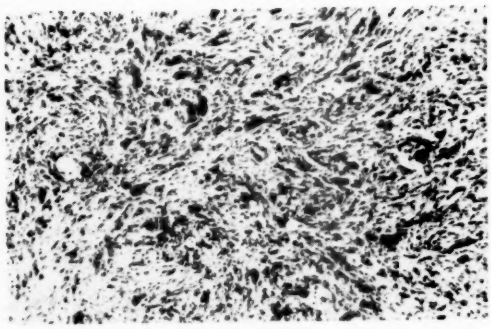

Fig. 1. Specimen taken at first curettage of Case No. 5: The tumor cells are rather loosely arranged and giant cells containing a swarm of nuclei each are seen among them. The cells show little atypism and have only little intercellular substance. (H.-E. staining.)

Fig. 2. Amputation specimen of the same case as above: Cell proliferation is advanced and syncytism is also evident. Mitotic pictures abundant and the tumor giant cells much reduced in size from those in Fig. 1 above. (H.-E. staining.)

Fig. 3. Curettage specimen from Case No. 3: Syncytial arrangement of tumor cells and appearance of oligonucleated giant cells to be noted. (H.-E. staining.)

Fig. 4. Amputation specimen from Case No. 3: The tumor cells show polymorphism suggestive of the malignancy of the tumor. (H.-E. staining.)

Fig. 5. Drill biopsy specimen from Case No. 9: The tumor cells are polymorphous and a few of them are in the course of mitosis. (H.-E. staining.)

Fig. 6. Amputation specimen from Case No. 9: The tumor cells show strong tendency of multinucleated giant cell formation, but giant cells with less numerous nuclei are also abundantly in presence. Besides, giant cells of malignant type and cells showing atypical mitosis are also in evidence, and the case was diagnosed as having a frankly malignant tumor. (H.-E. staining.)

Fig. 7. Specimen of lung metastasis from Case No. 9: Numerous multinucleated giant cells are contained giving the picture of polymorphous cell sarcoma closely resembling the primary focus. (H.-E. staining.)

Fig. 8. Excised specimen from Case No. 20: Malignant giant cell tumor of bone showing atypical mitosis and giant cells of malignant type. (H.-E. staining.)

Fig. 9. Excised specimen from Case No. 2: Marked but circumscribed fibre hyperplasy, poor in tumor giant cells, conspicuous in some parts of the tumor tissues. (Masson's staining.)

Fig. 10 and Fig. 11. Amputation specimen from Case No. 23: The behavior of argyrophil fibres towards the giant cells in formation shows so much difference in different parts of the same preparation. (Silver impregnated.)

Fig. 12. A so-called non-osteogenic fibroma (curettage specimen): Fibrous stroma in active formation, and numerous multinucleated giant cells are mingled in the stroma. The tumor cells showing no atypism, showing that the tumor is of benign nature. Such a picture may probably appear when an osteogenic fibroma is associated with giant cell tumor tissue. (H.-E. staining)

The Tohoku Journal of Experimental Medicine, Vol. 71, No. 4, 1960

Spectral Response Curves and Receptive Fields of Pre- and Postgeniculate Fibres of the Cat

By

Hisao Suzuki, Norio Taira and **Koiti Motokawa**
(鈴木 寿夫) (平 則夫) (本川 弘一)

From the Physiological Laboratory, Tohoku University, Sendai

(Received for publication, November 20, 1959)

Introduction

All the visual message from the retina to the central nervous system must be sent through the optic nerve. Thus, if the signal is intercepted at the level of the optic nerve fibre or the retinal ganglion cell from which the optic nerve fibre originates, it would not be impossible to reveal all the aspects of the visual stimulus such as its brightness, colour and shape. Adrian and Matthews[1] worked on the whole optic nerve of the Conger eel and found that information about the intensity of illumination was signalled by the frequency of impulses. This was confirmed by Hartline and Graham[2] in the single optic nerve fibre of the *Limulus* eye. Granit[3][4] carried out extensive investigations on the retinal mechanism of colour vision, but did not touch upon its conduction mechanism. Donner[5] maintained that information about wave-lengths is signalled in the form of temporal patterns of impulses. Lennox[6] recorded action potentials of single optic nerve fibres of the cat in response to various coloured lights and could correlate their conduction velocity to colour sensitivity. The laminar structure of the lateral geniculate bodies (LGB) of the monkey and cat was investigated by Glees and Le Gross Clark[7] and by Glees.[8] It was hypothesized by Le Gross Clark and Chacko[9] that the laminar structure may be interpreted in the sense of Young-Helmholtz's theory of colour vision. Cohn[10] recorded single unit discharges in the LGB of the cat in response to coloured lights, but obtained no element specifically sensitive to coloured light. In the experiment by De Valois, Smith, Karoly and Kitai[11] the part of the LGB of the monkey receiving the projection from the fovea was sensitive to red light, while the part receiving the projection from the retinal periphery was sensitive to blue light. As is well known, Granit classified retinal elements into dominators and modulators, which subserve luminosity and colour vision respectively, but

it is not clear whether or not the same classification holds good for elements in the central nervous system, because the optic nerve fibres and the fibres in the optic radiation do not make one to one correspondence; it is reported by O'Leary[12] that in the dorsal nucleus of the LGB of the cat each principal cell makes synaptic contacts with terminals of several different optic nerve fibres within the same layer. In the present investigation spectral reponse curves were constructed with unit discharges at both the optic tract and optic radiation as indicators in order to confirm Granit's result and to get information of possible modification at the LGB.

The receptive field of the optic nerve fibre was first investigated by Hartline[13] in the frog, and then by Kuffler[14] in the cat. However no information is yet available about the receptive field of the postgeniculate fibre. In the present experiments receptive fields for both single optic nerve fibres and those of the optic radiation were mapped by means of an automatic device to be mentioned below.

Methods

Experiments were performed on adult cats. For surgical procedures the animal was initially narcotized with ether and then cannulation of the trachea was performed. When necessary a small dose of pentobarbital sodium was injected through an inlying cannula in the saphenous vein. The head of the animal was fixed by a special head-holder convenient for exploration of the visual field. The marginal and suprasylvian gyri were exposed as usual. To minimize the respiratory and pulsatile movements of the brain the posterior cistern was opened. For recording from the optic tract, the dorsal surface of the LGB was exposed so as to allow the insertion of an electrode into the optic tract under visual control. The exposure of the LGB was achieved by aspirating the marginal and suprasylvian gyri and the dorsal hippocampus.

Time was allowed for the animal to recover from narcosis, and then the experiment was started. During the experiment the animal was paralysed with D-tubocurarine. Respiration was maintained mechanically through the cannulated trachea. Fall in temperature of the exposed brain was prevented by keeping the room temperature above 30°C.

The pupil of the eye to be illuminated was dilated with a few drops of 1% atropine sulfate solution. The cornea was protected from drying with saline. The cornea of the other eye was covered with a black disc fitted to the conjunctival sac.

For recording unit discharges, a 5 M-NaCl glass micropipette with ohmic resistance of 10–30 MΩ was used in most cases, but in some cases a 3 M-KCl glass micropipette with ohmic resistance of similar order was also used. A silver plate placed on the frontal sinus served as an indif-

ferent electrode.

In Fig. 1 is shown schematically the general arrangement of the recording and stimulating equipments for obtaining spectral response curves.

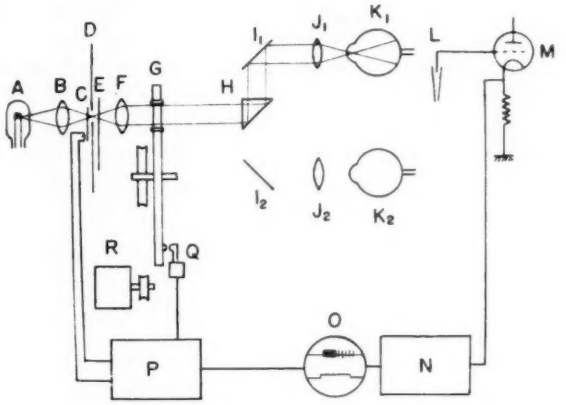

Fig. 1. Schema of general arrangement of recording and stimulating equipments for obtaining spectral response curves. Explanation in text.

The light source A was 200-watt (100 V, A.C.) tungsten filament lamp for a projector which was lit at a mean colour temperature of 2400°C. The filament was focused onto the slit D by a lens B. A shutter C for controlling the period and timing of illumination was placed just in front of the slit, and the opening and closing of the shutter were controlled electronically by an electronic pulse generator with a delay circuit P. The light from the slit was made parallel by a collecting lens F and finally focused onto the pupil of the right eye K_1 by a collecting lens J_1 via a reflecting prism H and a plane mirror I_1 to cause diffuse illumination of the retina. The light beam could be focused onto the pupil of the other eye K_2 by a lens J_2 via a mirror I_2 after rotating the prism H. The light beam was about 20 mm. in diameter and measured 150–200 lux on a plane perpendicular to it. The intensity of the light was controlled by means of neutral filters E placed behind the slit. Monochromatic lights were obtained by the use of interference filters G and were adjusted to equal energy by suitable neutral filters. The wave-length ranged from 400 mμ to 680 mμ in steps of 20 mμ. The filters G were arranged on the circumference of a large circular disc so that the light beam passed successive filters while the disc was revolving. Illumination of 300 msec. duration was repeated at an interval of 2 sec. Search for unit discharges was carried out with white light of 15–20 lux. The output of the glass micropipette L was led to an

RC amplifier N with a cathode follower input stage M. The output of the amplifier was led to one beam of a double beam cathode-ray oscilloscope O, the other beam being used for recording the time mark and period of illumination. When the isolation of unitary discharge was satisfactory, the disc with the interference filters was set in motion by a motor R to explore the spectral response of the unit. When each filter came to a right position, a microswitch Q was pressed automatically, and thus the sweep of the beams of the oscilloscope was triggered. The shutter was then opened with an adequate timing by the delay circuit.

For exploration of the receptive field an automatic device was employed which is shown schematically in Fig. 2. Basically it consisted of

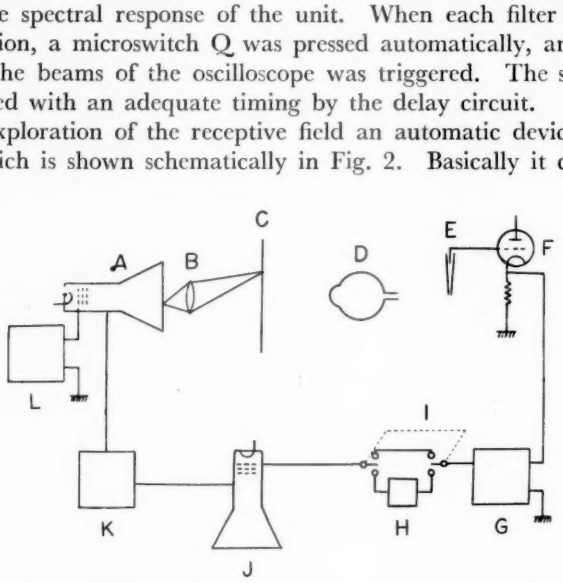

Fig. 2. Schema of automatic device for mapping receptive fields. See text.

two cathode-ray tubes, one for obtaining an exploring light spot and the other for producing scintillations corresponding to nerve impulses. A television tube A of 14 inches in diameter made an exploring light of sufficient intensity available, and a cathode-ray tube J of 6 inches in diameter served for the production of scintillation. The beams of both tubes were swept synchronously in the horizontal direction by a common scanning generator K. The light spot was 2° in visual angle and projected onto a screen C measuring 40 cm. in diameter and made of white paper with average transmission of about 4%. At a distance of 20 cm. behind the screen the eye D to be explored was placed so that the screen subtended 90° in visual angle. The light spot was flickering at a rate of 1.8 cps. and moved horizontally at a constant rate of about 3 cm./sec., the duration of each flash being about 30 msec. The input signal of the glass micropipette E was amplified by an RC amplifier G with a cathode follower input

stage F and led to the brightness modulation circuit of the oscilloscope J. When a light spot fell on a point within the receptive field of a single optic nerve fibre the brightness of the beam was so modulated by the impulses in response to the light spot that scintillations corresponding to the impulses were produced at the place on the cathode-ray screen which corresponded to the locus of the light spot. The horizontal scanning was repeated with a period of about 15 sec., the beam being displaced vertically by a certain definite step each time. In this way the visual field of 90° in diameter could be scanned by exploring spot in about 230 sec. Thus, the receptive field was mapped as an assembly of bright spots on the screen of J. During the scanning it occurred sometimes that the amplitude of impulses changed for unknown reasons, and it was possible that these changes in amplitude would cause distortion of the receptive field. This draw-back was, however, avoided by the use of a trigger circuit H which served to keep the brightness of scintillation constant.

All experiments on receptive fields were carried out under light adaptation. As the source of adapting light was used a projector, the light of which was projected onto the screen C. The intensity of the adapting light was controlled by a step-down transformer and rarely exceeded 8 lux on the screen.

By direct measurement with an excised cat's eye it was found that visual fields of 20°, 40° and 60° corresponded to circles of 3.4, 6.7 and 13.3 mm. in diameter on the retina respectively.

Results

Discharge patterns

For recording unit discharges at the optic tract an electrode was inserted into the entrance of the optic tract to the LGB. Well isolated unit spikes were positive monophasic and measured a few millivolts in most cases. Judging from location of the tip of the microelectrode and the shape of spikes[15] they seemed to originate from a single optic tract fibre. Examples of their three discharge types, "on", "on-off" and "off", obtained in response to white light of 300 msec. duration and 15–20 lux are represented on the left in Fig. 3. In any type of response impulse discharges began with relatively high frequency and declined thereafter in the rate. The decay was rapid especially with the "on" component of the "on-off" type unit. The discharge type in a given optic tract fibre seemed to be fixed as far as diffuse illumination of the retina was concerned; no change in discharge type occurred over a wide range of intensity and wave-lengths, as is exemplified in Figs. 4 and 6.

For recording unit discharges at the optic radiation a glass micropipette

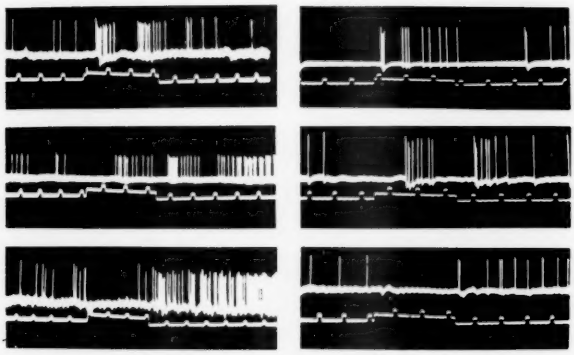

Fig. 3. Discharge patterns obtained from single optic tract fibres (left column) and from single optic radiation fibres (right column) under dark adaptation. Upper row: " on " type, middle: " on-off " type, lower: " off " type. In this and subsequent figures upward deflection of upper beam denotes positivity, lower beam showing time mark and period of illumination. Time marker, 100 msec.

was inserted deeply into the primary visual area. Positive monophasic spikes measuring a few millivolts and responsive to light were obtained usually at a depth of 2–4 mm. beneath the cortical surface. It was much more difficult to isolate a unit responsive to illumination in the intracortical layer. The depth at which the unit discharges were isolated and their shape[15] suggested that they were obtained from a single optic radiation fibre.

The response from the optic radiation fibre could be classified also into three types, " on ", " on-off " and " off ", as is exemplified on the right in Fig. 3. It seemed that the frequency of spikes and their whole number were generally small as compared with discharges of the optic nerve fibre obtained under comparable stimulus conditions. This was a constantly observable finding, although its physiological significance is not clear.

Relation between the intensity of illumination and the discharge rate

In the following experiments intensity dependence of discharge rates at pre- and postgeniculate levels was studied. The dependence was most simple with the " on " type unit at the pregeniculate level; the relation was sigmoid in shape when the average frequency within the first 300 or 600 msec. from the onset of illumination was plotted against log intensity of illumination. The maximum frequency was as high as 100 cps. in

most cases. The " off " component of the " on-off " unit showed a similar dependence, but the " on " component showed no clear-cut dependence, as is illustrated in Fig. 4 in which the average frequency within 300 msec.

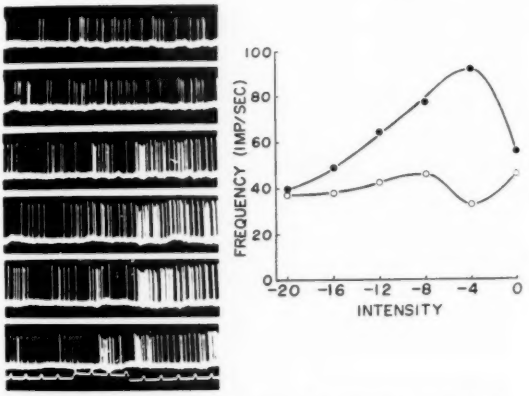

Fig. 4. Relation between intensity of illumination and discharge rate of single optic tract fibre of " on-off " type in response to white light of 300 msec. duration. Records from top to bottom correspond to relative intensity of 0.7^{20}–7.0^{0} in steps of 0.7^{4} respectively. In graph on right average frequency of impulses was plotted against log relative intensity of illumination. Average frequency of impulses was calculated from impulses within period of illumination for " on " component of response (open circles) and from those within first 300 msec. after cessation of illumination for " off " component (filled circles). Time marker, 100 msec.

from cessation of illumination is plotted as ordinate. In this case the frequency of impulses reached a maximum (87 cps.) at a relative intensity of 0.7^{4} and decreased with further increase of intensity, as was observed in Hartline's experiment.[13] It is to be noted that the discharge of the " on " component suffered from some reduction in accordance with the frequency maximum of the " off " response. In the response of the " on-off " unit such inhibition at a certain intensity of illumination was a usual finding. The " off " type unit behaved very much like the " off " component of the " on-off " unit against changes in intensity of illumination.

In Fig. 4 at relative intensity of 0.7^{20} the discharge rates of the " on " and " off " components coincided, and this rate of 40 cps. is nothing but the rate of the spontaneous discharge. Most units of the " on " and " off " types showed spontaneous discharges of 30–40 cps., but it was observed that some units of the " off " type fired spontaneously at a rate as high as 70 cps.[16]

What has been said about pregeniculate fibres holds good for post-

geniculate ones. An example of " on-off " units is shown in Fig. 5. As can be seen in this figure, the highest frequency of the postgeniculate fibre was about 30–40 cps., the spontaneous discharge rate being about 10–20 cps. Although isolation of " off " type units available for such systematic measurements was unsuccessful, the same may hold for this type of response.

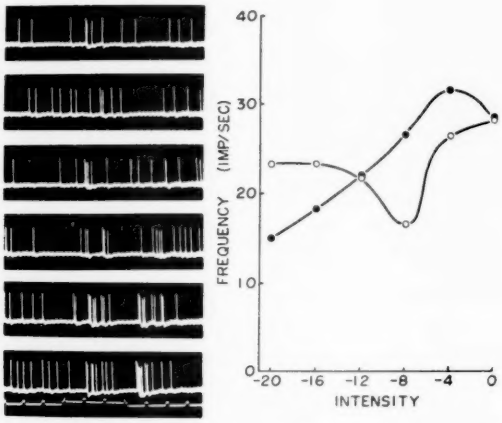

Fig. 5. Relation between intensity of illumination and average frequency of " on-off " type unit in optic radiation. Open and filled circles refer to " on " and " off " components respectively. Time marker, 100 msec.

As clear in the above findings, the output of the LGB was definitely reduced in both discharge frequency of response and that of spontaneous activity as compared with the input. However, similarity was found to be preserved beyond geniculate synapses as to the relation between the intensity of illumination and the discharge rate.

Spectral response curves

At the pregeniculate level both response of the " on " type unit and that of the " off " type unit gave spectral sensitivity curves with a maximum at 480–500 mμ under dark adaptation. In most responses of the " on-off " type unit, the " off " component alone showed a similar curve. An example is illustrated in Fig. 6 in which the spectral sensitivity curve is represented by a heavy line connecting threshold responses for the " off " component. These curves seemed to accord fairly well with the scotopic dominator curve by Granit.[17)18)]

Similar results were obtained with optic radiation fibres and cortical neurones (see Figs. 7 and 8). The response at the cortical level illustrated

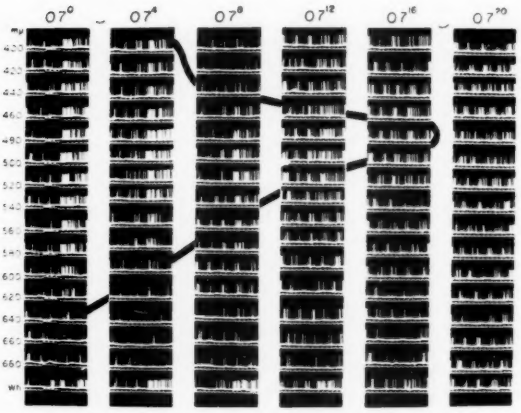

Fig. 6. Spectral sensitivity curve of single optic tract fibre (" on-off " type). Time marker, 100 msec.

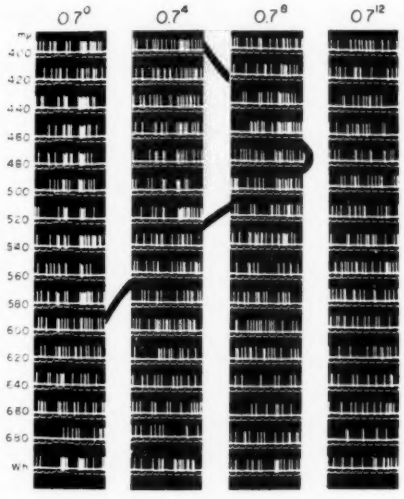

Fig. 7. Spectral sensitivity curve of single optic radiation fibre (" on-off " type). Time marker, 100 msec.

in Fig. 8 needs some comments. The response was assigned to a single cortical neurone for the following reasons: 1. It was obtained at a depth of 2.3 mm. from the cortical surface. 2. The rate of spontaneous discharge was decidedly low as compared with that of fibres. 3. The response occurred in the form of a brief burst. 4. The spike was diphasic (positive-

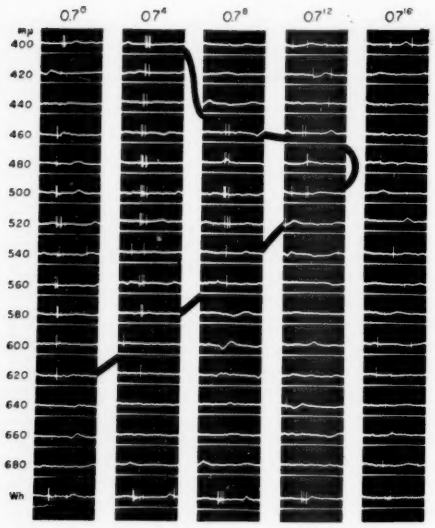

Fig. 8. Spectral sensitivity curve of single neurone in visual cortex. Time marker, 10 msec.

negative) instead of monophasic.

In order to investigate the behaviour of response against wave-lengths of coloured lights of equal energy, a kind of spectral response curve was constructed by plotting the average frequency of impulses at both pre- and postgeniculate levels against wave-lengths. At the optic tract such curves obtained with coloured lights of strong intensity showed either a broad maximum at about 500 mμ or a plateau extending over a very wide range of wave-lengths except at the red end of the spectrum at which the curves fell abruptly. When the intensity of coloured lights was reduced to a certain level, these curves fell off towards both ends of the spectrum, giving a peak at about 500 mμ. Thus, the curves obtained at low intensity levels resembled the spectral sensitivity curves mentioned above. These changes in spectral response curves due to alterations in intensity were common to any type of response, as is shown in Fig. 9 in which curves of continuous line refer to a relative intensity of 0.7^6. At the postgeniculate level spectral responses to strong coloured lights were of more complicated form. The average frequency of impulses was decidedly low at about 500 mμ and two maxima appeared over the range of 420–480 mμ and the range of 520–600 mμ. These two maxima disappeared on reducing the intensity of measuring coloured lights, and, instead, a maximum appeared at about 500 mμ. Such dependence of the response curve on the intensity

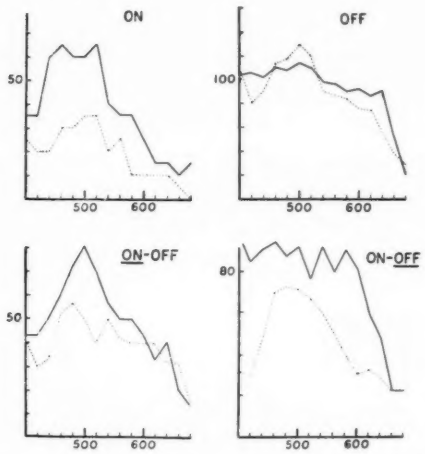

Fig. 9. Spectral response curves of single optic tract fibres. Ordinate: average frequency of impulses. Abscissa: wave-lengths in mμ. Continuous curves were obtained with strong lights of 0.7^0 and dotted ones with weak lights of 0.7^6. Type of discharge taken as indicator is underlined.

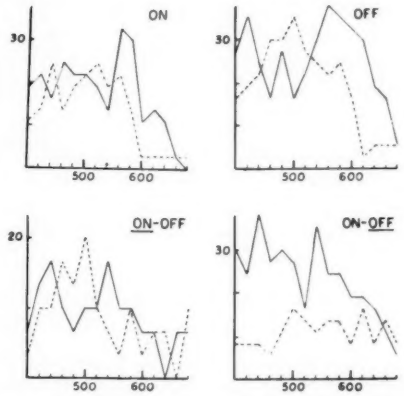

Fig. 10. Spectral response curves of single optic radiation fibres.

of measuring lights or adaptation level could be noticed for all response types, as is shown in Fig. 10.

The difference between the response curves obtained at the pre- and postgeniculate levels consists in that the photopic and scotopic characters of response curves may be distinguished more clearly at the postgeniculate level.

Receptive field

The receptive field of a single pregeniculate fibre was investigated at various adaptation levels. In Fig. 11 receptive fields of a pure " off " unit are illustrated. The test of the response type was carried out with a diffuse flash of white light of about 1 msec. duration. Under dark adaptation no definite receptive field could be mapped owing to spontaneous background discharges (see record labelled 0). When spontaneous discharges were reduced by background illumination, the receptive field could be mapped as an assembly of light spots, which was roughly circular in shape. As the intensity of adapting light was increased, the receptive field was reduced progressively in size and disappeared (see records labelled 16, 18, 19, 21 and 22). The smallest receptive field obtained in this experiment was 3.5 mm. in diameter which corresponds to 20° in visual angle. This size of receptive field was comparable with that obtained by Kuffler[14] in the cat and with that by Thomson[19] in the rabbit.

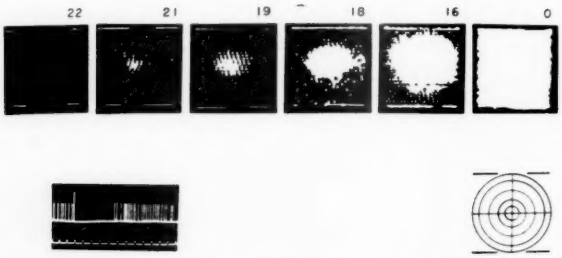

Fig. 11. Receptive fields of single optic tract fibre of " off " type. Relative intensity of adapting light is given above each receptive field. " Off " discharge pattern of fibre obtained under dark adaptation is shown on left below. Concentric circles on right below measure 20°, 40°, 60°, 80° and 90° in diameter respectively.

What has been said above held generally for " on-off " units as well. However, it is to be noted that the receptive fields of some " on-off " units decreased and then increased in size, as adaptation level was progressively raised. An example is shown in Fig. 12. The contour of the receptive field obtained at higher adapting levels was so irregular that it appeared like a starfish as a whole. The mechanism of this phenomenon will be discussed later.

The receptive field of a pure " on " unit could be mapped with certainty only under dark adaptation, because in the presence of adapting light the unit showed maintained discharge which obliterated the contrast between its response to the test light and the background.

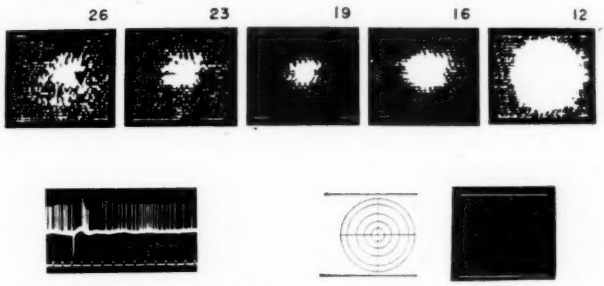

Fig. 12. Receptive fields of single optic tract fibre of " on-off " type.

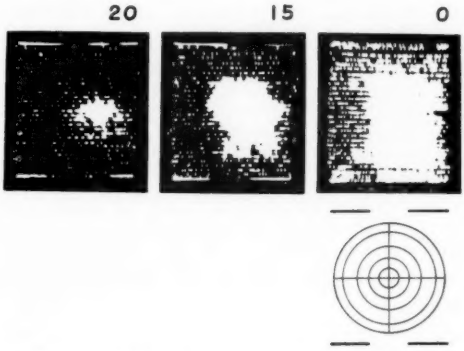

Fig. 13. Receptive fields of single optic radiation fibre of " on-off " type.

At the postgeniculate level similar results were obtained. In Fig. 13 receptive fields of an " on-off " unit are illustrated. As the number of cases was not sufficiently large with respect to postgeniculate fibres, it must be decided in further investigation, whether or not there is any quantitative difference between receptive fields for pre- and postgeniculate fibres.

Discussion

The fact that the output of the LGB in terms of discharge rate is generally smaller than the input has a parallel in other sensory pathways studied by previous investigators.[20][21]

Scotopic spectral sensitivity curves obtained at three levels of the visual pathway, the optic tract, optic radiation and primary visual cortex, are essentially the same with a maximum at about 500 mμ. They correspond to Granit's scotopic dominator and may be a neurophysiological counterpart of the spectral sensitivity curve determined behaviourally by Gunter.[22] At any level no spectral sensitivity curve corresponding to any one of

Granit's modulators could be obtained under dark adaptation.

With stronger coloured lights some response curves extending over a relatively narrow range of wave-lengths were obtained at the postgeniculate level. They seemed to have two maxima, one at shorter wave-lengths than 500 mμ and the other at longer wave-lengths. Further investigation, neurophysiological as well as behavioural, would be needed to decide whether or not the double peaks in the response curve suggest dichromatic photopic vision of the cat. Whatever the interpretation of spectral response curves may be, it must be emphasized that the colour response may be revealed more easily at the postgeniculate level.

The receptive fields of most units contracted with increasing intensities of adapting light. Attenuation of the effect of scattered light at raised adaptation levels may be one of the factors responsible for the contraction. Another factor conceivable would be development of an inhibitory zone arround the receptive field; when inhibition is not so strong the response —say " on " response—will be inhibited within this zone, but no response of the other type—say " off " response—will appear yet. In consequence the receptive field will be smaller in size when moderate inhibition prevails than otherwise. When inhibition is sufficiently strong, responses of the other type will appear in the inhibitory zone. Since both " on " and " off " responses contribute to mapping the receptive field in the present technique, the inhibitory zone will be included into the receptive field; thus the receptive field will become larger again when the intensity of adapting light is raised above a certain level. Fig. 12 illustrates such an example. Development of an inhibitory surround was shown by Kuffler[14] and further by Barlow, FitzHugh and Kuffler.[23]

Summary

Unit discharges were recorded from the optic tract, optic radiation and visual cortex of the cat immobilized with D-tubocurarine.

1. Three response types, " on ", " on-off " and " off ", were distinguished at pre- and postgeniculate levels. The discharge rate was found to be decidedly low at the postgeniculate level as compared with that at the pregeniculate.

2. The relation between the average frequency of impulses and log intensity of illumination was generally sigmoid in shape, but the " on " component of most " on-off " units did not show any remarkable dependence on the intensity. This component was peculiar also in that it showed a frequency minimum at a certain intensity.

3. Scotopic spectral sensitivity curves obtained at three levels of the visual pathway were almost identical, having a maximum at about 500 mμ.

4. Average-frequencies of impulses plotted against wave-lengths or spectral response curves were found different from the spectral sensitivity curves, especially at higher levels of intensity of illumination. The difference was greater at the postgeniculate level, where the curve consisted of two elevations extending over 420–480 mμ on the one hand and over 520–600 mμ on the other.

5. The receptive fields of pre- and postgeniculate single fibres were mapped by means of an automatic device. The receptive fields of most units, pre- or postgeniculate, contracted as the intensity of background illumination was raised, but in some "on-off" units an expansion of the receptive field was observed above a certain level of adaptation.

References

1) Adrian, E. D. & Matthews, R., J. Physiol., 1927, **63**, 378.
2) Hartline, H. K. & Graham, C. H., J. cell. comp. Physiol., 1932, **1**, 277.
3) Granit, R., Sensory mechanisms of the retina. Oxford Univ. Press, 1947.
4) Granit, R., Ergebn. Physiol., 1950, **46**, 31.
5) Donner, K. O., Acta physiol. scand., 1950, **21**, Suppl., 72.
6) Lennox, M. A., J. Neurophysiol., 1958, **21**, 70.
7) Glees, P. & Clark, W. E. Le Gross, J. Anat., Lond., 1941, **75**, 295.
8) Glees, P., *ibid.*, 1941, **75**, 434.
9) Clark, W. E. Le Gross & Chacko, L., Nature, Lond., 1947, **160**, 123.
10) Cohn, R., J. Neurophysiol., 1956, **19**, 416.
11) De Valois, R. L., Smith, C. J., Karoly, A. J. & Kitai, S. T., J. comp. physiol. Psychol., 1958, **51**, 662.
12) O'Leary, J. L., J. comp. Neurol., 1940, **73**, 405.
13) Hartline, H. K., Amer. J. Physiol., 1938, **121**, 400.
14) Kuffler, S. W., J. Neurophysiol., 1953, **16**, 37.
15) Tasaki, I., Polley, E. H. & Orrego, F., *ibid.*, 1954, **17**, 454.
16) Kuffler, S. W., FitzHugh, R. & Barlow, H. B., J. gen. Physiol., 1957, **40**, 683.
17) Granit, R., Acta physiol. scand., 1943, **5**, 219.
18) Granit, R., J. Neurophysiol., 1945, **8**, 195.
19) Thomson, L. C., J. Physiol., 1953, **119**, 191.
20) Katsuki, Y., Sumi, T., Uchiyama, H. & Watanabe, T., J. Neurophysiol., 1958, **21**, 569.
21) Katsuki, Y., Watanabe, T. & Maruyama, N., *ibid.*, 1959, **22**, 343.
22) Gunter, R., J. Physiol., 1952, **118**, 395.
23) Barlow, H. B., FitzHugh, R. & Kuffler, S. W., *ibid.*, 1957, **137**, 338.